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Fig. 1.—A Typical Modern Microscope.—Made by W. Watson and Sons to the specification of Dr. Henri Van Heurck, Antwerp, for Photo-Micrographic and High Power work.

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MODERN MICROSCOPY

A Handbook for Beginners and Students

COMBINING

I. THE MICROSCOPE, AND INSTRUCTIONS FOR ITS USE

BY

M. I. CROSS

II. MICROSCOPIC OBJECTS: HOW PREPARED AND MOUNTED

BY

MARTIN J. COLE

LECTURER IN HISTOLOGY AT COOKE'S SCHOOL OF ANATOMY

THIRD EDITION

ENTIRELY REVISED AND ENLARGED

TO WHICH IS ADDED

III. MICROTOMES: THEIR CHOICE AND USE

LONDON
BAILLIÈRE, TINDALL AND COX
8, HENRIETTA STREET, COVENT GARDEN
1903

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3. J. 101.

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PREFACE TO THIRD EDITION.

An entire revision of the text has been made in this new edition. Information regarding the microscope, its accessories and methods of manipulation, have been brought into line with present-day knowledge and practice.

The instructions for preparing and mounting microscopic objects have been extended and are more varied.

A third part has been added on the subject of the choice and use of microtomes, for which we desire to express our special thanks to Mr. G. West, of the Botanical Laboratory, Edinburgh. The directions given are compressed within narrow limits, and are designed especially to aid the beginner. Acknowledgments are also due to the publishers of *Knowledge*, of 326, High Holborn, for permission to use several notes and articles which have appeared in their columns, and to numerous friends who have contributed their kind advice and suggestions for the improvement of the book.

M. I. C.
M. J. C.

London,
November, 1902.

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PREFACE TO FIRST EDITION.

THIS handbook is not intended to be an exhaustive treatise on the microscope, nor to give particulars of the various patterns of instruments that are made, of which details can be seen in the makers' catalogues, but to afford such information and advice as will assist the novice in choosing his microscope and accessories, and direct him in his initial acquaintance with the way to use it.

The directions for preparing microscopic objects by Mr. Martin J. Cole are the outcome of a very long experience as a preparer of Microscopic Objects of the highest class, and cannot fail to be of the greatest service to the working microscopist.

M. I. CROSS.

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MODERN MICROSCOPY

INTRODUCTION.

To attempt to give a historical account of the development of the microscope, tracing it through its pre-achromatic days, and noting the successive improvements that have contributed to the high position it occupies to-day amongst instruments of precision, would alone fill a small volume. Suffice it to say that so recently as the year 1824 Tully constructed his first Achromatic Microscope, since which time every decade has added its tale to the march of progress. In 1883 the President of the American Society of Microscopists, in his annual address, remarked 'that lenses, which were believed to have so nearly reached the limit of perfection fifteen years ago, are antiquated now, and the limit of perfection has moved forward like the horizon, and is as far off as ever.' Those who are acquainted with the development of the microscope and its appurtenances since that time are aware that greater progress has been attained in recent years than had ever been made before. With the introduction of the system of lenses, termed the 'apochromatic objectives,' microscopical optics were raised to an entirely new plane, for these lenses were of a nature altogether superior to those of their predecessors. Subsequently a new and increased variety of optical glasses from which to manufacture their lenses was placed at the disposal of opticians, and from that time

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the leading manufacturers have vied with each other in their attempts to provide inexpensive objectives that have some of the most important qualities associated with apochromatism, particularly freedom from spherical aberration; in so high a degree have they been successful that the so-called students' series of objectives by many makers, at what may be considered nominal prices, are in many instances superior to the finest lenses of twenty years ago. This improvement in the construction and performance of the objective has brought in its train complementary developments in the sub-stage condenser, and necessarily increased accuracy and improved design in the mechanical construction of the microscope-stand; and any worker who is interested in the scientific and artistic sides of microscopy can view with nothing but intense satisfaction the distinctly progressive nature of everything connected with the instrument itself and the absorbing secrets it reveals. That this onward march will continue is certain, for the issues which depend on the microscope for their solution are ever increasing in number. The demands that are made on it are more exacting and varied than ever before, and there will always be forthcoming the willing brain to devise facilities to meet them.

The principal factors which have contributed to the progress that has taken place in microscope construction, optical and mechanical, are: Firstly, the unceasing criticism, impartial examination, and, where merited, recommendation, together with the suggestion fraught of mature knowledge, skill, and thoughtful consideration on the part of amateur microscopists, and especially a select number whose names are familiar to every worker with the microscope; secondly, to the exacting requirements of modern bacteriological research, medical work generally, its adoption in numerous branches of education, and in manufacturing industries of every description.

Not many years ago a hospital rarely possessed more than one or two microscopes, and these were usually kept

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under glass shades, and intended more for ornament than use. At the present time every hospital student has to provide himself with a microscope, and become practically acquainted with the ultimate structures of organs and tissues, both in health and disease. The microscope has been introduced into many ordinary schools, and teachers are slowly realizing that 'we think in pictures,' and that as sight aids the memory, so an ounce of ocular demonstration is worth a pound of oral description. The result is, that erstwhile dry-as-dust lectures in zoology and botany are now anticipated by students with keen interest on account of the pleasure derived by examination with the microscope of the actual subject of study. By means of the microscope the pharmacist is able to judge of the quality of his drugs; the Medical Officer of Health uses it to detect adulterations, entozoa, and bacteria; the brewer and the baker watch their ferments with it, and it is employed by iron and steel workers, seedsmen, dairymen, clothworkers, handwriting experts, and, in fact, in nearly every vocation it is becoming more and more an indispensable referee. It therefore is something more than a mere tool which magnifies, for it contributes in a vital degree to our well-being and comfort.

To the microscopical societies also the evolution of the microscope is due in no small degree, and especially does this apply to the Royal Microscopical Society and the Quekett Microscopical Club, both of which meet in London at 20, Hanover Square. Every improvement in the instrument and its accessories that takes place is presented to these societies for criticism, and in connection with both of them, as officers and members, are men who have attained the highest eminence in microscopical science and manipulation, whose judgments have influenced and moulded the character of microscopy, and who are ever willing to assist by advice and suggestion any who will avail themselves of their experience. It is most desirable that microscopists should become members of a good microscopical society,

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and those just mentioned enjoy the highest position in England.

Undoubtedly the professional worker has in a large degree to thank the amateur microscopist for the efficiency of the microscope that is to-day available, for he it is that has devoted time and money to the encouragement of the manufacturer.

It is surprising that, notwithstanding the pleasures and advantages that are associated with microscopical work, microscopy has not the hold on people of refined tastes that its merits should fairly claim for it. It does not seem to be realized that the microscope will unfold its wonders and beauties without that long and careful study which is the necessary preliminary to the majority of scientific pursuits. Those who may be induced to use a microscope in the first place for pleasure and recreation will quickly find their inclinations leading them to a desire for fuller knowledge concerning the subjects which may come under their notice, and by degrees this instrument will become the means of the acquirement of a very liberal education, for its influence is not merely confined to one kingdom; it embraces every tangible and intangible subject, whether it be the air we breathe, with its myriads of invisible friends and foes to human well-being, or the floors of oceans, with their minute flinty shells bearing markings which exceed in accuracy the power of any draughtsman to depict, and which in themselves are invisible to the naked eye, many of them measuring but the $\frac{1}{1000}$ of an inch.

Astronomy, with all the wonders that are associated with the study, demands many a night vigil, an expensive instrument, and a suitable observatory, and even then there is always a sense of dissatisfaction when accounts are read of observations of fellow-workers who are more highly favoured in that they possess glasses of very large aperture, which the average astronomical student could not aspire to. How dependent the observer is on the weather, too!

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Photography, with its manifold uses and the pleasant memories associated with numerous pictures that are secured, cannot be compared with the microscope, more especially for the long winter evenings.

For any kind of recreation to produce the mental rest which is required by the man of business, a fresh set of faculties must be brought into play, and no better method can be imagined for the purpose than the introduction to a world whose variety is illimitable, whose form is lovely and unique, and whose subjects can never be met with excepting in the quiet observations through the microscope-tube in the study.

Perhaps some people may hesitate to attempt working with the microscope, not caring to use it merely as a means of amusement, and mistrusting their ability to employ it scientifically. They reflect that every department has its untiring, experienced workers, and available ground appears to have been gone over so repeatedly that it would seem hopeless for an amateur to attempt to add to existing knowledge on any subject. This idea is a mistaken one, and any microscopist who uses his instrument thoughtfully will be surprised at the manner in which the love for the work will grow upon him, and how gradually he will become master of some special department which he has adopted as his own. On this point we would echo the words of a well-known microscopist: 'It needs no marvellous intellect, no special brilliancy, to succeed in a scientific study; work at it ardently and perseveringly, and success will follow.'

In order that the best results may be obtained, however, there must be a correct understanding of the methods of working the instrument. Facility in this respect can only be acquired as the result of experience and practice, and it is the object of this work to indicate in the plainest manner the rules of manipulation that should be adopted in order to insure success.

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PART I.

CHAPTER I.

THE MICROSCOPE-STAND.

As one looks through the catalogues of the various dealers, and notices the microscope-stands varying in price from £2 to £40, a feeling of bewilderment arises as to what is essential and what can be dispensed with. We will, then, examine the parts, describe their uses and advantages, and state what is necessary for a beginner.

Here let us advise intending purchasers not to buy a microscope unless it bear the name of a manufacturer: a good workman is never ashamed of his handiwork. There are many very inferior instruments that look tempting, but a practical acquaintance with them soon discovers their weak points and inefficiency. Happening to attend the conversazione of a well-known microscopical society, at which there were exhibited over one hundred instruments, it was surprising to note the many makeshifts of microscopes belonging to some of the exhibitors—and many of them had probably cost a fair price, too. A manufacturer once remarked to the writer that he was some time ago in a provincial town, when an auctioneer asked him whether he could make him up a job lot of microscopes for sale by auction, as he was very successful in disposing of a certain class of pictures in that way. The disgust of the scientific workman can be better imagined than described. The microscopes often seen in the novice's possession seem to be

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of this genus, and but little satisfaction is derivable from working with them. If the user is at all progressive, an instrument of this kind is either speedily discarded in favour of a well-made one, or it may, on the other hand, cause him to become disheartened, and attribute want of success to his own incapacity instead of the poor quality of the instrument.

Although a good second-hand instrument may be occasionally met with, great discretion is required in purchasing, because improvements may have been introduced since its manufacture, or some damage may have occurred to the optical parts. If it be obtained from a respectable dealer who understands his business, and will give a guarantee of condition, there is some inducement; but a friend who is up to date in microscopy is generally the best to advise. In all cases before purchasing, a catalogue should be obtained from the maker whose name the instrument bears, so that it may be ascertained whether the pattern is still made, or is antiquated and out of date. It is much better to buy a good stand, capable and worthy of receiving additional apparatus from time to time, rather than an inferior instrument that is completely furnished with objectives and accessories. These latter rarely engender pride of ownership, and are often relegated to some obscure corner after a short acquaintance; whereas, if a good instrument be purchased, with but one objective to start with, there is always a pleasure in working with it, and a peculiar fascination from its quality—a satisfaction in feeling that one has something superior.

On page 8 a typical student's microscope is figured, by reference to which the different parts of the instrument will be made clear.

Fig. 1.—A is the stand or foot.

B is the tailpiece carrying the mirror (C) with which light is thrown upon the object.

D is the under-fitting, into which are fitted the sub-stage condenser, polarizer, etc.

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FIG. 1.—A TYPICAL STUDENT'S MICROSCOPE.
The 'Fram' Microscope. By W. Watson and Sons.

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E is the stage on which the object is placed.

F is the limb carrying the body (G).

At the lower end of the body is a nosepiece (H), having a screw into which the objective is fitted.

At the upper end of the body is a sliding fitting called the draw-tube (J), by means of which additional magnification may be obtained, and into this draw-tube the eyepiece or ocular (K) fits.

L is a rackwork, by means of which the body (G) is raised and lowered in order to focus the objective upon the object which is placed on the stage (E).

M is the milled head controlling the fine adjustment, which imparts a delicate motion to the body, in order that the objective may be more exactly adjusted than would be possible with the rackwork (L) when using high magnifying power.

N is a fitting for forceps, or side silver reflector; and O are the springs with which the object is held in position.

We have selected the instrument (Fig. 1) because, from practical acquaintance with it, we are able to strongly recommend it for a beginner's microscope, worthy of receiving additions from time to time as means may permit. Still, it should only be considered as a typical one.

THE FOOT.

Since the first edition of this book was issued, a decided reaction has taken place in regard to the form of foot on which the microscope is mounted. There has scarcely been a modern writer of repute who has not urged the necessity and importance of having such a mounting for the microscope as shall secure for it absolute rigidity, whether it be used vertically, inclined, or horizontally for photography. No foot so fully answers these requirements as the tripod pattern. Rarely does it happen that the bench or table on which work is done is absolutely level, and the tripod is the only pattern that naturally adjusts

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itself to such inequalities of surface. It cannot, therefore, be too emphatically insisted that microscopists should select this pattern in preference to any other. At first sight this feature may appear to be a somewhat trivial one, and especially so to a novice, yet minor details have a marked significance in the satisfactory execution of his work. It will be found advantageous to have a foot shod with cork, as thereby the microscope is in a degree insulated from vibration, and the risk of scratching the surface of the table on which it is being used is avoided. It must be clearly understood, however, that even this form of foot must be made in correct proportion, or its advantages will be annulled.

Next to this foot in point of rigidity we would place the Jackson model, as shown fitted to the instrument on pages 37 and 41. This, when properly made, though somewhat heavier, is but little inferior to the tripod.

Of variants from these two designs there are many, but their rigidity can be tested by placing the instrument in a horizontal position, racking the body out, and then observing if there be a tendency to topple over; if there be, have nothing to do with it.

We have purposely left the type of foot known as the 'horseshoe' to the last, because we consider it the least advantageous of all. It has enjoyed and still commands a distinct preference on the part of a very large section of workers all over the world, and that section the one that uses the microscope to a greater extent than all others put together—we refer to medical students and medical laboratories generally. It is to be remembered that a great deal of work in laboratories is done with the instrument in an upright position, in which circumstances the condenser and mirrors are perhaps somewhat more easily manipulated with a horseshoe foot and pillar than with the tripod.

It is claimed also for the horseshoe foot that it is more compact, and that if it be unconsciously brought beyond

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the front edge of the bench it will remain firm, where a tripod instrument would fall completely over. These are just demands.

While an instrument mounted on the horseshoe is in a vertical position it stands firmly; but if the body be inclined at an angle, as an amateur usually employs it, even with a large and heavy foot there is a tendency to side-falling. All considered, the tripod has so many advantageous points that it is to be recommended in preference to any other. Our selection of a foot for a microscope would therefore be in the following order:

1. The tripod foot, as shown fitted to instruments on pages 8, 25, 32, and frontispiece.

2. The Jackson form of foot, as fitted to the instrument illustrated on pages 37 and 41.

3. The Continental or horseshoe form, as shown on page 12.

THE STAGE.

The stage of the microscope on which the object is placed for examination may be divided into two classes: (1) mechanical, and (2) plain.

The Mechanical Stage. — The instruments figured on page 25 and frontispiece are provided with this type of stage, in which, by the turning of two milled heads which are attached to screws, plates are moved in dove-tailed grooves one over the other, in rectangular directions, carrying the object with them. A first-class microscope should be provided with this form of stage; in fact, there is no means so suitable for systematically examining an object as is afforded by it. In addition to these mechanical movements, if a bar be fitted to slide in a vertical direction on the top plate the efficiency of the stage will be greatly increased. The mechanical stage lends itself to the adaptation of further important movements. A means of rotating the object is an essential in most classes of work. For this purpose the lowest plate of the stage is usually fitted to rotate on the

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FIG. 2.—TYPICAL CONTINENTAL MICROSCOPE.
Stand I, by E. Leitz, Wetzlar. (Showing attachable mechanical stage.)

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fixed centre of the base-plate, the mechanical movements acting above it. It is then termed a concentric rotating stage, the object remaining in the field during the whole rotation of the stage. In mechanical stages of economical construction the rotating plate is occasionally fitted above the mechanical movements, and is carried by them, in which case it does not rotate concentrically. The object can, notwithstanding, be kept in the centre of the field by constantly re-setting it with the mechanical screws during the rotation of the plate. Some stages of the concentric form are arranged to rotate by rackwork and pinion; although this is not really an essential, it is often convenient; it also prevents the stage from rotating accidentally, especially in photography. When it is provided, it should have the pinion-wheel so arranged that it may be disengaged from the rack and replaced instantly.

Centring screws to the concentric rotating stage, by means of which the axis of the stage may be made true with any objective, will be found a useful addition, especially if petrological work is to be done. Divisions to the periphery of the stage for reading the angle through which the stage is rotated are not advantageous for ordinary purposes, but for chemical and petrological work they are a necessity.

Finders to Mechanical Stages.—Divided scales, reading to parts of an inch or millimetre, fitted to the plates of the mechanical stage, will be found of great utility. By means of such an arrangement, important parts of an object can be noted and subsequently refound. For instance, supposing a specimen were being examined, and an important feature were observed to which future reference would be desirable, it would only be needful to take the reading of the divisions on the stage, and record them on the slide—say, horizontal, 24; vertical, 20. On future occasions, on setting the stage readings at the same points and placing the object in the same position on the stage (for which purpose nearly all mechanical stages have a

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stop-pin, against which the slide can be set), the special feature would be at once in the field of view. These divisions can also be used for roughly measuring objects, the *modus operandi* of which is given in the instructions for the measurement of objects, page 114.

If a mechanical stage be selected, it should be a good one, for if badly made it is far less convenient than a plain stage; also the frictional parts should be sprung, and fitted with adjusting screws, so that compensation may be made for wear and tear.

Attachable Mechanical Stages. — In recent years a variety of mechanical stages, which can be attached to or removed from an ordinary plain stage microscope, have been introduced. Some of these possess merit, but, taken as a whole, they are inaccurate in working, and at their best are not for one moment to be compared with the mechanical stage, which has been built as an integral part of the microscope, and no microscopist who wishes to do himself and his work full justice should entertain such a fitting.

For some reason, Continental manufacturers have never fitted their instruments with the mechanical stage as it is understood in England, but have always recommended and arranged for the adaptation of an attachable stage. Where mechanical movements are found to be an essential for certain work, and from reasons of inconvenience or impracticability the plain stage cannot be exchanged for a proper mechanical one, then, and then only, should the attachable form be resorted to.

Plain Stages.—The stage of the microscope shown on page 8 has two flat springs only, to hold the object in position on the surface, and the movement of the object is effected by the fingers. For cursory examinations this answers every purpose; but where systematic work is to be done something more is needed, and this, when a mechanical stage is not provided, should take the form of a bar reaching completely across the stage and sliding in a

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vertical direction. If properly fitted and sprung, it will travel freely when gently pressed with one hand only. The object is carried by it, and can be moved in a horizontal direction upon this bar. With a little practice the fingers become educated to the work, enabling examinations to be conducted with the highest powers almost as rapidly and systematically as with the mechanical screws. This sliding-bar should further be provided with two flat springs, so fitted that they may be turned inwards to rest on the bar when not required. It is often necessary to set an object at an angle across the stage during observation, in order that some special feature may appear vertically in the centre of the field. If the springs be not provided this cannot be done.

Finders for Plain Stages.—The form of finder suggested by Mr. Lewis Wright for plain stages is the most efficient for practical purposes. Many proposals have been made, but none equal this one for simplicity. On the right-hand side of the central aperture, one inch of the stage is divided into 50 parts in vertical and horizontal directions. A special feature of interest in an object having been discovered, the slide being maintained in a horizontal position across the stage by means of the sliding-bar, it is only necessary to read from the top right-hand corner of the slide the lines against which it lies. A note of same is made on the labels of the object, and the specimen can subsequently be placed in exactly the same position, and the subject re-examined. Without the sliding-bar it is somewhat difficult to keep the object exactly straight across the stage, but with care, on observing an important feature, the slide can be gently turned until it is in a correct position for taking readings.

The great saving of time that is afforded by such a device as this should establish its claim to be placed on every student's microscope. Several makers have already adopted the arrangement, and it would be a great gain to microscopists in general if a uniform position for the divisions

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were agreed upon between them, so that a person noting a special point with his microscope could send the specimen, with the readings marked upon it, to a brother worker, and he, having the same kind of finder on his stage, would at once be able to find the desired spot.

The following method would be suitable for the average size of stage: A piece of metal the same size as an ordinary glass slip (3 × 1 inches) should be adopted as a tool, and $\frac{3}{4}$ inch from one end and $\frac{1}{32}$ inch from the edge a minute spot should be made with a small drill. The metal slide should be placed on the stage with the spot towards the front, and the $\frac{3}{4}$-inch space to the right of the centre of the stage. The drilled spot should then be placed central in the field of a 1-inch objective, and the outer margin of the square of divisions marked off from the right-hand end of the metal slide.

The Wright's finder is obviously unsuitable for any other than a stage whose upper surface does not travel.

In selecting a stage for a microscope our choice would therefore be as follows:

For a first-class microscope: Mechanical movements; concentric rotation; screws to make the rotation quite true with any objective; sliding-bar to top plate and stop-pin for object to go against; divisions to plates of stage reading to parts of millimetre or inch; rackwork rotation to stage; and (optional) divisions to periphery of stage.

For a second-class microscope: Mechanical movements; sliding-bar to top plate; non-concentric rotation.

For a third-class microscope: Plain stage, with springs to hold object in position; if provided with sliding-bar or plate as object-carrier, so much the better.

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THE SUB-STAGE OR FITTING UNDER THE STAGE TO CARRY CONDENSER, ETC.

The Sub-stage.—This consists of a tube which should be 1·527 inches = 38·786 millimetres, or, roughly 1½ inches full, internal diameter—termed the 'Society's size.' It carries illuminating apparatus for condensing the light on the object, the polarizing prism, and other apparatus, referred to on a later page. It is adjusted in a vertical direction to and from the under surface of the stage by means of a rack and pinion, and the ring carrying the apparatus is mounted in an outer collar provided with screws, by means of which the condenser, or other apparatus, can be made exactly central with the objective with which it is working. This central fitting is made to rotate by rack and pinion in some instances for using the polarizer, etc., but this is so rarely needed that it is hardly necessary except for special work. It is essential that the sub-stage should be substantially made, as it is a most important fitting, often too little appreciated. For the sake of economy some makers fit the sub-stage without a rack-work, it merely being designed to slide up and down in the dovetailed fitting; this is neither desirable nor convenient. A fine adjustment, to permit of the condenser being focussed in the most exact manner, is often provided with the best stands, and it is exceedingly convenient and of very great importance where high-power work is intended to be done. Often it is wished just to alter the focus of the sub-stage condenser very slightly. In attempting so to do the tension on the milled head of the rack-work is apt to cause vibration, so that the best point of adjustment cannot be at once observed. By communicating this small amount of motion with the fine adjustment the focus is obtained to a nicety. It is also especially convenient where a number of specimens have to be examined. The varied thicknesses of the slips necessitate a slight readjustment of the condenser in each

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instance, and this can be very quickly done if a fine adjustment be fitted to the sub-stage. Further, the modern sub-stage condensers possess such large apertures that their exact adjustment becomes equal in importance to the precise focusing of the objective.

Where a microscope is provided with a sub-stage it is necessary to ascertain if it will centre with the objective by means of its screws; this should be done in the same manner as described below for the 'under-fitting,' and the centring screws turned. Also, it is very important that when the sub-stage is racked up or down it should maintain its centre with the optical axis. But few instruments will stand this test; in consequence of untrue mounting or build the sub-stage goes out of centre—slightly in some cases, considerably in others. There ought to be absolute truth if everything is square, and any great deviation in this respect should call for rectification. If a fine adjustment be fitted to the sub-stage, it may be tested by using the upper surface of the fitting as a stage and placing the object on it; this may be made to adhere with a little tallow or grease. An objective of medium power will probably not focus, the sub-stage being too far away. The nosepiece end of the microscope must therefore be lengthened; for this purpose remove the prism from an analyzer fitting, and use this fitting as a lengthening adapter. The object is then viewed in the usual way.

In the construction of the sub-stage once again the Continental microscopes are not for a moment to be compared with their English contemporaries. There is not a single maker of microscopes on the Continent who provides centring screws to his sub-stage. It is simply impossible to do good work without this convenience. There is hardly a worker nowadays who does not have objectives by more than one maker, and it will be found that these have different centres. Continental opticians maintain that their method simplifies work, for the condenser is centred once for all to the objectives that are supplied with the instruments.

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Simple and obvious tests will quickly demonstrate that this is quite fallacious.

THE UNDER-FITTING.—In the cheaper instruments, instead of the sub-stage as above described, an ordinary plain tube, termed the under-fitting, is screwed into the under side of the stage, and in this tube the condenser or other apparatus is moved up and down to focus. It is shown fitted to the microscope figured on page 8. This must be

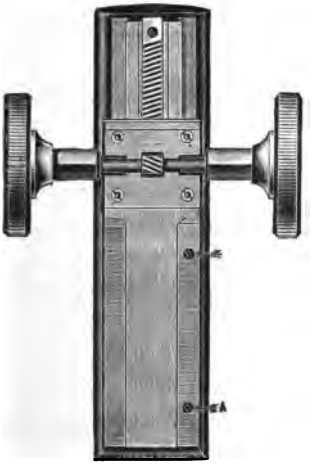

FIG. 3.—VIEW OF DIAGONAL RACKWORK ATTACHED TO ONE OF WATSON AND SONS' MICROSCOPES, SHOWING DOVETAILED FITTINGS TO RECEIVE FINE ADJUSTMENT SLIDE AND ADJUSTING SCREWS (MARKED A).

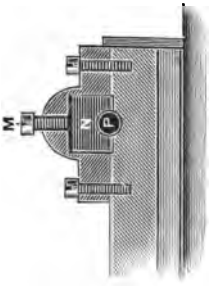

FIG. 4.—SECTIONAL VIEW OF ADJUSTABLE FITTINGS OF RACK AND PINION.

truly centred with the optical tube, and it is well to test it by placing a small diaphragm in the under-fitting, and with an objective in the body to focus the diaphragm. If it be not central its practical importance is annulled. The additional convenience and necessity of the centring sub-stage cannot be too fully impressed upon the beginner who contemplates doing thorough work. Swift and Son, of Tottenham Court Road, in their new series of 'Students' Microscopes,' and Watson and Sons, of High Holborn, in

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their 'Edinburgh Students' and 'Fram' Instruments, make many of the fittings interchangeable, so that a person who starts with an under-fitting, and subsequently experiences the necessity of having a sub-stage, can himself remove one fitting and replace it by the other. W. Watson and Sons have lately adopted a form of centring under-fitting; it is inexpensive and very considerably increases the efficiency of a student's microscope. It can be fitted to almost any microscope.

The great convenience will be found in many instruments of being able to swing the sub-stage aside out of the optical axis of the instrument on a hinge-joint fitting. It saves much time to students, especially where two or three powers are constantly being interchanged, and the condenser may not be required for all of them. Where this arrangement exists it should be adapted in a workman-like and substantial manner, and a proper support given to the fitting when in the optical axis to make it perfectly rigid.

The choice with regard to a sub-stage would therefore be—

In a first-class microscope: Sub-stage, having rack-work and fine adjustment for focusing, and provided with facilities for centring; rackwork rotation, if for examination of crystals or for petrology.

Second-class instrument: Sub-stage, having rack-work and centring adjustments, and means of lifting aside out of the optical axis.

Student's instrument: The same as the second-class, or with the plain under-fitting; the latter, preferably with centring arrangement. In any case it is imperative that it shall be of the 'Universal' size.

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ARRANGEMENTS FOR FOCUSING.

The next point for consideration is the means of focusing the object-glass. This is done by two adjustments, called the coarse and the fine movements. The former consists of a rack and pinion actuating the body in a very true-fitting dovetailed bearing, as per Fig. 3 shown on page 19. In the illustration it will be seen that the rack is cut diagonally, and this is undoubtedly the best kind, a softer motion being obtainable than with the horizontal form. In order that it may work at its best, each tooth of the rack has to be carefully 'ground in'—that is, fitted to a leaf in the pinion—and so that the fitted tooth of the rack may always engage the correct leaf of the pinion, it is necessary so to fix the body that, when racked up as high as possible, it may not be withdrawn from its bearings and rackwork, it being, in fact, provided with a 'stop' screw. The pinion should have suitable provision by means of adjusting screws for exactly controlling the stiffness of the rackwork action and for taking up slight backlash which may arise in consequence of wear and tear. An illustration of the method adopted to secure this result is shown in Fig. 4, page 19, in which the pinion P is held in position by a block of metal, N, against which pressure is exerted by two screws, one of which, M, is shown in the figure. With this arrangement the most exact relation of pinion to rack can be established and maintained.

It has been recommended that microscopists should take their instruments to pieces in order that they may judge of their workmanship; but in reality a well-made microscope requires to be as carefully put together as a watch, and for a novice to attempt to undo the parts means very probable detriment to the instrument. The name of a first-class maker on an instrument may generally be considered a guarantee of good workmanship, otherwise he could not possibly maintain his reputation.

Some cheap students' microscopes, instead of being pro-

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vided with a rack and pinion for the coarse adjustment of the object-glass, are made with the body to slide in a fixed tube. This is a very rough-and-ready arrangement, and accuracy of centring cannot be maintained as with a rack and pinion. These instruments are, however, largely used in hospitals and medical schools, and possessing one element of advantage—namely, cheapness—other considerations are often made subsidiary. Instruments of this kind are generally cast aside or disposed of in favour of an instrument having rack and pinion after a very short time, and anyone purchasing a microscope with a view of adding apparatus to it would be well advised in having one with a little less apparatus, but with a rack and pinion instead of a sliding body.

FINE ADJUSTMENTS.

Well-defined attempts have been made by nearly all makers to improve this most important of all movements. The demand for accuracy in this particular has been greatly increased by the growing use of objectives of large aperture which cannot be profitably employed excepting with a fine adjustment of the utmost precision. Its essentials are that it impart a very slow motion and be absolutely free from lateral movement. The fine adjustment that for many years has proved thoroughly satisfactory in the writer's hands is that made by Messrs. Watson and Sons and applied to all their instruments. It is shown in position on the instrument (Fig. 1) and the working details will be gleaned from Fig. 5.

The body is raised or lowered in a dovetailed fitting by means of a lever contained within the limb of the instrument, and a pin passing through it transversely acts as a fulcrum. By turning a milled head attached to a micrometer screw, force is applied to the lever at one end against a pointed rod, attached to the body and entwined by a coil spring, at the other extremity. As the body moves upwards, the spring is compressed against a brass plate, and on the

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micrometer screw being released this spring produces the reactionary power. One arm of the lever is four and a half times longer than the other, consequently the weight of the body at the milled-head end of the lever and the motion imparted are reduced in this ratio. Thus the makers give the weight of a body of one of their instruments as 17 ounces, and this divided by $4\frac{1}{2}$ reduces the resistance to $3\frac{7}{9}$ ounces. This system has the advantage that the

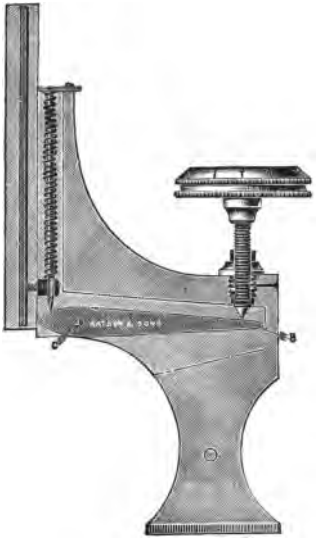

FIG. 5.—SECTION OF LIMB OF MICROSCOPE TO SHOW SYSTEM OF FINE ADJUSTMENT.

position of the milled head on the limb is convenient for manipulation, and is not altered when the body is racked up—that is, it is not carried by the rack-work, as in many forms, so that its attachment to a focusing rod of a camera for photo-micrography is easy and convenient. There is also a very simple means of adjustment provided for taking up any slackness through wear. The slide in which the fine adjustment is fitted has sprung slots, to which are fitted screws (shown in Fig. 3, page 19, marked A). By turning these screws slightly, the spring-fitting grips the

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bearing more tightly, and so takes up any wear caused by friction. Any microscopist can thereby adjust his own instrument.

This pattern of fine adjustment in modified forms is now made by at least two other houses. It would not be applicable to microscopes other than those in which the so-called Jackson limb is employed, and makers of stands in which a direct acting screw is fitted, as in the Continental form, have exercised considerable ingenuity in providing an efficient movement. Carl Reichert, of Vienna, who has in recent years shown highly progressive tendencies, designed a lever-form of fine adjustment for this latter description of microscope, which has proved even more satisfactory than was originally anticipated. Messrs. Swift and Son, too, have adopted a somewhat similar plan with corresponding success, while Messrs. Zeiss have devised an entirely distinct fine adjustment for their photographic stand, actuated by means of gear-wheels, which produce an extremely slow and precise movement.

Powell and Lealand's instruments are also provided with a fine adjustment having special merit, consisting of a lever actuating a long tube sliding up and down inside the body. This, however, has the disadvantage of being carried by the rack-work when focusing for different powers.

Two-speed Fine Adjustments.—Complaint has arisen that the tendency to fit very slow-acting fine adjustments has become a source of inconvenience to students and others who work interchangeably with objectives of different powers fitted on revolving nose-pieces. To meet this Mr. A. Ashe, of the Quekett Club, designed a two-speed fine adjustment, and the plan was carried to a practical issue by Messrs. R. and J. Beck, who now apply it to certain of their instruments. It is shown fitted to the microscope (illustrated page 25). It will be noticed that two milled heads are provided for fine focusing instead of the usual one. The upper milled head turns a screw having a coarse thread, moving the body $\frac{1}{60}$ inch for each revolution; the lower

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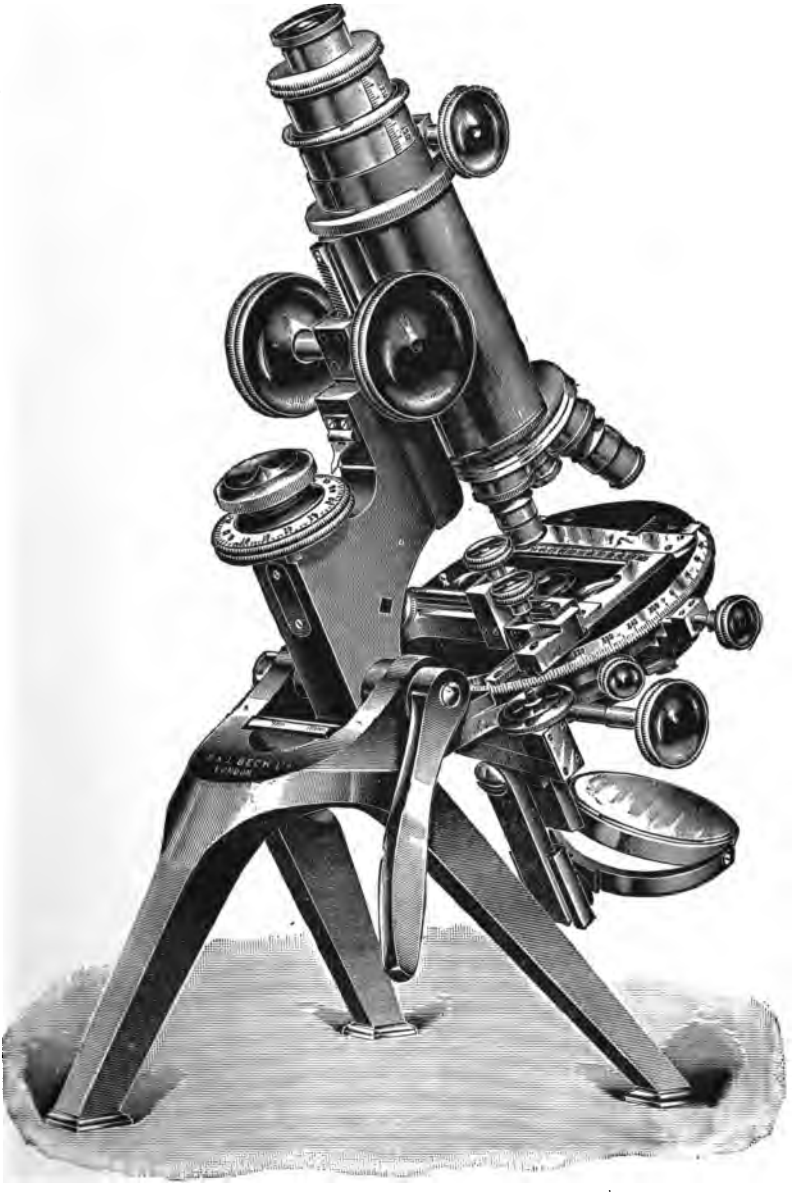

FIG. 6.—'IMPERIAL' MICROSCOPE BY R. AND J. BECK.
(Showing two-speed fine adjustment.)

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actuates a fine screw which causes a movement of the body $\frac{1}{300}$ inch for each complete turn. At any moment either milled head may be used and time thereby saved when low powers are employed.

Where a simpler method may be desired, practically the same result can be secured by having attached to the centre of the ordinary milled-head a smaller spindle, say, one-sixth of the diameter of the former. When a quick movement is required this spindle can be turned between the fingers, and a rate equal to six to one of that obtainable by slowly rotating the ordinary milled-head will be secured. Messrs. Watson and Sons have applied this to their instruments, and the writer has found the working exceedingly satisfactory.

When testing the performance of the fine adjustment, a central cone of light must be used; if the light be thrown obliquely there will be of necessity an apparent movement in the direction from which the light comes. With central illumination there should be no shake or displacement whatever in the object when it is focused.

Nearly every maker has his own system or systems of fine adjustment, possessing features more or less desirable, but they are mostly modifications of those mentioned here. Some firms adopt a most excellent form of fine adjustment for a superior class of microscope, while in the students' patterns the method employed is dissimilar and oftentimes useless for high-class work. It would be far better that efficiency were not sacrificed in such a manner for the small saving in cost involved.

FIG. 7. — SPINDLE MILLED HEAD FOR GIVING TWO SPEEDS TO FINE ADJUSTMENT.

Above all things avoid the form of fine adjustment which carries the *whole* weight of the body of the instrument, or depresses it against a spring, as in the Continental instruments and cheap students' forms: these are almost worse than no fine adjustment at all, as they invariably soon work loose in the fittings and cause great annoyance.

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There is also another form, now almost non-existent, which is fitted to the microscope at the nosepiece end, and consists of a milled head attached to the body, by means of which a tube which carries the objective is raised and lowered inside the body-tube against a spring. This can never work thoroughly well, for if two round tubes are perfectly fitted one inside the other, they clutch, and to overcome this, one of the tubes has to be rendered a little eccentric, and consequently a lateral shake arises.

In the choice of a fine adjustment, therefore, reject the direct acting and the nosepiece forms.

THE LIMB.

The design of the limb of the microscope is of special importance, because it carries the body and is intimately associated with the fine adjustment. It should be of a substantial shape and strongly made. In our opinion the pattern which is shown in the build of microscopes, pages 8, 25, etc., is to be preferred before that shown on page 12, because in the former, additional solidity is imparted to the body fittings on account of there being no separate adjustment which has to act and re-act at the back part—in other words, a limb which carries the body at one extremity, and at the other is acted upon by the fine adjustment through a pillar, cannot in the nature of things be so satisfactory as a limb which carries the fine adjustment instead of being supported by it. Still, it cannot be denied that the method of attaching the limb to the pillar adopted by the majority of the Continental makers, and, for the matter of that, those English manufacturers who include this style of instrument amongst their models, is usually a very substantial one.

THE BODY-TUBE.

It has always been the custom, in the construction of the full-sized English microscopes, to make the body of fairly large diameter; while on the Continent the reverse is the

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case, and it is made as small as possible. Owing to the extended use of Continental oculars and objectives, English makers have in recent years adopted the Continental diameter of body to a considerable extent. For photographic purposes it is held by some workers that a fairly large body should be used, but for ordinary visual work we do not consider there is any real advantage in the large over the small tube. The growing system of providing a large outer body with an inner draw-tube to carry the small students' eyepieces, is a commendable step, and meets all needs. It may here be said that instruments of Continental type have their bodies constructed very much shorter than the so-called English models, the rule being to employ objectives adjusted for a tube 160 millimetres long with the former, while English opticians usually adjust theirs to one of 250 millimetres. The microscopist who enters enthusiastically into his work invariably has objectives of both Continental and English make, and he therefore requires the convenience of being able to use both perfectly. One or two English opticians make microscopes with a body of 160 millimetres, and a draw-tube sliding inside it, by means of which a length of 250 millimetres can be obtained; but a greater range is often found convenient, and Watson and Sons in their Van Heurck microscope (shown in the frontispiece), and Mr. Baker in his Nelson model microscope, supply two draw-tubes giving a range of body from 140 millimetres ($5\frac{1}{2}$ inches) to 310 millimetres (12 inches). One of these draw-tubes works by a rack and pinion: the object of this being to afford facility for adjusting the objective for thickness of cover-glass, as described on page 68. This form of body is coming more and more into use, and will be found a very great convenience to the all-round worker. No precise advice can be given without knowing the work intended to be done, but, generally speaking, the short body with the two draw-tubes is much to be preferred to any other.

The draw-tube usually has a scale of divisions engraved

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upon it to parts of a centimetre or inch. The object of these divisions is to enable a record to be kept of magnifications at different points of extension, or a note to be made of the lengths of tube that give the most perfect corrections for certain objects and objectives.

In all microscopes of medium or high-class, the universal thread should be fitted to the lower end of the draw-tube; where there are two draw-tubes it should be supplied to the outer one. The advantages of this adapter are numerous. A low-power objective can be used in it which it is often impossible to focus on many stands, owing to the compactness of the build and shortness of the movement of the coarse adjustment. With the two draw-tubes, if the outer one have this adapter fitted, nearly 10 inches of separation can be obtained between the eyepiece and the objective. It is further useful for carrying the apertometer objective and the analyzer, described respectively on pages 63 and 102; also the Bertrand's lens for examining the 'brushes' of crystals, and for many other purposes.

It has occurred within the experience of the writer that results obtained on a microscope having a large tube could not be reproduced with the same objective on an instrument having a small tube. This was traced to be due to the diaphragm at the bottom of the draw-tube, and it has since been found that in many students' stands the opening of this diaphragm is as small as $\frac{3}{8}$ inch. This is altogether insufficient, and causes restriction to the passage of rays from the objective. It would be well to see that this diaphragm had an opening of at least $\frac{3}{4}$ inch.

TAILPIECE AND MIRRORS.

The Tailpiece.—It will usually be found convenient if the arm, or tailpiece, which carries the mirrors, be so mounted as to be turned aside with the mirrors when desired. This arrangement is of great utility, for it permits of light being readily directed from the lamp through the

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sub-stage condenser for critical work. Opticians favour a rectangular rather than cylindrical tailpiece to carry the mirror gymbal; the reason for this is a little doubtful, but there is probably no distinct advantage in one over the other. Where, however, a cylindrical tailpiece is provided, it will be obvious that the mirror could quickly be swung round out of the axis of the microscope and so obviate the necessity for the swinging of the tailpiece itself, but this is quite a minor consideration.

The Mirrors should be plane and concave, hung in a gymbal, giving universal movements, and have a means of adjustment to focus in a vertical direction. The plane mirror is always used with the condenser, spot lens, etc., and with very low-power objectives, but the concave, when the condenser is not employed and the maximum amount of light is desired.

A constant source of trouble and annoyance is an imperfectly worked plane mirror, which will give several reflections of the image of the lamp flame. The plane mirrors usually fitted invariably do this. It should be insisted, in a microscope with which high-class work is to be done, that a parallel-worked mirror should be supplied. It is a trifle more costly than the ordinary kind, but the additional efficiency, on account of the reduced number of reflections, is very appreciable. The parallelism of a mirror may be tested by holding it just below the level of the eye in the direction of a row of objects, such, for instance, as chimney-pots; and on observing the reflections, each subject should stand out singly and clearly. If the mirror is not parallel-worked several reflections of the same object will appear superimposed in the mirror.

Care is needful in the use of the concave mirror, if the best result is to be obtained with it. It should be so arranged that the apex of the cone of rays that it transmits may be exactly in focus on the object. Many microscopes are provided with mirrors that are unsuited to the instrument, being either too long or too short in focus, and con-

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sequently do not produce good effects. To test the mirror, a piece of white paper should be placed upon the stage of the instrument, which must be set horizontally, and light from a lamp reflected by the concave mirror on this; then, by sliding the mirror up and down on the tailpiece, it can quickly be seen if the focal point can be obtained upon the paper.

BINOCULAR MICROSCOPES.

We have hitherto been treating principally of the monocular microscope, and this, it must be understood, is the only form that can be used for critical high-power work —in fact, the Continental firms as a rule do not make binocular microscopes at all, regarding them as unnecessary. Two or three of them, however, make a binocular eyepiece, which will be found described under the head of eyepieces. The advantage of a binocular microscope is, that both eyes can be employed simultaneously, saving the strain on the vision which is apt to ensue through the constant employment of the monocular microscope, and the endeavour to see in the best manner the detail in the specimens examined. We should recommend every user of the monocular microscope to train himself to work with either eye, keeping the one not in use open; this will be found of the very greatest service. The universally understood binocular microscope is provided with a prism, designed by Wenham, which admits of the light going up a direct tube, and reflects light also into a second tube. By this means objects can be seen more naturally than with the monocular microscope, for the reason that stereoscopic vision is obtained, and objects having a certain amount of depth may be seen completely with the binocular microscope, whereas with the monocular it would be necessary to focus in successive stages through the entire depth. Especially is this true regarding opaque objects, with low powers. The stereoscopic binocular conveys an impression of the

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objects viewed that is almost startling in its beautiful effect. Subjects stand out in relief, exhibiting their natural contour, and at once the worker is able to decide the shape and form of an object in a way that it is impossible to do by focusing through the several planes with a monocular

FIG. 8.—A POPULAR BINOCULAR MICROSCOPE.
The Nelson model by C. Baker.

instrument. The binocular microscope is *par excellence* the instrument for the amateur. To him the beautiful appeals in a manner that it perforce cannot do to the scientific man, who, being intent on the pursuit of knowledge of some obscure point, has no time to notice, or if

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to notice, cannot linger to reflect upon the æsthetic aspect. In the examination of rotifers and other inhabitants of 'ponds and rock pools,' perhaps the most charming subjects that the microscope has ever revealed, the microscopist with a monocular instrument cannot possibly appreciate and interpret structure and movement in the same accurate manner that the binocular enables him to do. These facts should receive careful consideration when a microscope is to be chosen, but it must be borne in mind that the Wenham stereoscopic form of binocular cannot be used with an objective having a higher air angle than 40°. Provision is, however, always made whereby the prism may be withdrawn, and the light then only goes up the monocular or straight tube, and the instrument is to all intents and purposes as useful and convenient as the monocular microscope, while the unemployed eye of the observer is rested by looking into the blank binocular tube; the fact of its not being illuminated will scarcely be noticeable.

For use with the binocular microscope, the closer the posterior lens of the objective is brought to the prism the better. In fact, some makers have constructed objectives in very short mounts, working quite on to the prism, up to a power of $\frac{1}{8}$ inch. These, however, are not recommended. Dr. Carpenter some time since pointed out that when an objective having an air angle exceeding 40° was employed with the Wenham binocular, spherical objects became distorted, and instead of appearing round in shape they became conical. The special apparent advantage gained is therefore useless.

Special high-power prisms are also made by two or three opticians, whereby higher powers may be employed with a binocular microscope; but we doubt whether the results obtainable with them are really worth the additional outlay. In those we have seen there has been such a very unequal illumination in the two tubes, that, personally, we should prefer using the instrument monocularly. It must be understood that all vision through the microscope in the

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ordinary way is inverted, that is, the object is seen upside down. A very good form of binocular microscope, devised by Stephenson and made by Swift and Son, erects the image, or, in other words, enables it to be seen the right way up. It is excellent for dissecting purposes, and high powers can be employed with it; still, it cannot be described as an all-round microscope, and would have to be classed with instruments for special work. Our advice on the question of a monocular or binocular microscope is: If the instrument be required for strictly educational, scientific, or photographic work, the monocular must be chosen. The bulk of the general amateur's work is done with comparatively low powers, and in such cases the binocular is unquestionably of advantage, and to be preferred. If it is proposed to combine scientific with general work, a good plan is to have two separate bodies—monocular and binocular—interchangeable in the same bearings. The maximum facility is then at the disposal of the user. It should be noted that when the two bodies are chosen, it is well to have centring screws to the rotating stage, as described page 13, because the bodies rarely have identically the same centres, and the stage could not otherwise be made to rotate concentrically with both bodies.

MICROSCOPES FOR SPECIAL PURPOSES.

Dissecting Microscopes.—The dissecting microscope is usually, so far as the stand itself is concerned, of plain, but substantial construction. It should have a good clear stage, preferably consisting of a glass plate, which can be interchanged with opal, glass, ebonite, etc., substantial supports for the hands; rack-work and pinion to focus the magnifying-lens; and a gymbal, carrying a mirror on one side and a mat opal disc on the other. In this department Continental manufactures may be considered superior to the English on account of their variety of design, substantial and excellent construction, and economy in price.

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We figure one by Leitz, which for all ordinary work can be recommended; in addition to this the same maker supplies

FIG. 9.—SIMPLE DISSECTING MICROSCOPE.
By E. Leitz.
The supports for the hands are attached to the two small buttons shown on the edge of the stage.

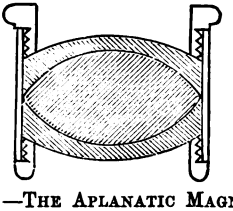

FIG. 10.—THE APLANATIC MAGNIFIER.

an erected image dissecting microscope and a series of excellently corrected *loupes*, or aplanatic magnifiers after

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Steinheil. Zeiss also makes most excellent dissecting microscopes and well corrected magnifiers. Dissecting microscopes of various patterns may also be found in the opticians' catalogues. For many purposes a magnifier mounted on a plain stand with an extending arm, known as lens-holders or magnifying-stands, will be found very useful.

It is eminently desirable that the magnifiers employed be of the aplanatized form, consisting usually of three lenses cemented together, similar to Fig. 10, p. 35. Many of them can be had so arranged in the mounting that they can be used either with a dissecting microscope or be carried in a suitable fitting in the pocket.

Microscopes for Metallurgical Work.—Examination of metal by means of the microscope is a comparatively modern study, but there is probably no iron or steel works of standing that is not equipped with suitable instruments both for observing and photographing. By means of the microscope much information regarding both the chemical constitution and mechanical properties are disclosed, but it is especially valuable for the latter. For instance, the structure of steel varies with the degrees of hardness and the amount of heat to which it has been subjected, and it is possible readily to gain definite information concerning the suitability of the metal for the purposes to which it is to be put by means of the microscope. In the manufacture of guns any defect which may have taken place in the heating or quenching of the steel, which would render the gun unsafe or unsatisfactory, can be discovered before the manufacture is proceeded with. Engineers can detect flaws, blow-holes, defective welds, etc., at an early stage, and avoid the trouble incident to the use of imperfect metal in the finished article.

Microscopes for this exclusive purpose require no sub-stage or mirror, and although it is customary to employ an ordinary microscope, so that the use may not be restricted to the one especial purpose, two or three well-designed

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instruments are manufactured for this work alone. We illustrate one by C. Baker on page 37, which has the following features:

The main support of the stage is carried in a dovetailed

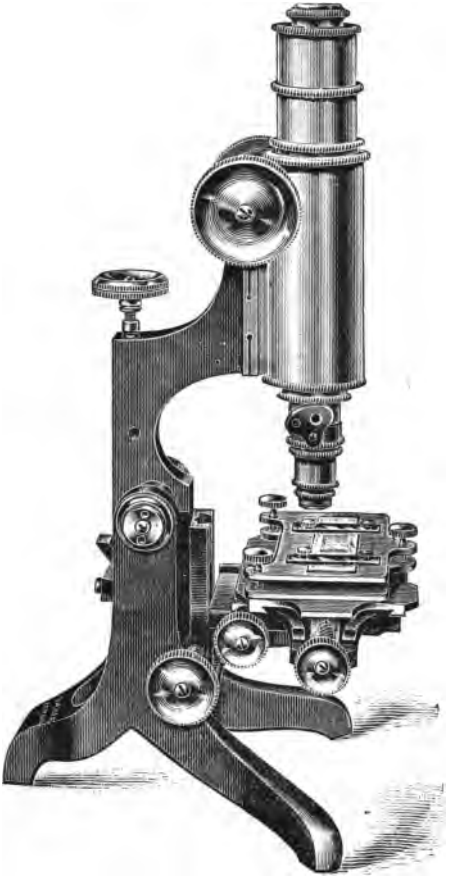

FIG. 11.—METALLURGICAL MICROSCOPE.
By C. Baker.

fitting, parallel with the body of the instrument, and can be raised or lowered by means of a rack-work and pinion.

The stage itself has on the upper surface a levelling-plate on which the specimen for examination is laid.

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Three screws permit of any want of parallelism between the faces of the specimen being compensated for.

To illuminate a specimen a vertical illuminator, the construction and use of which is referred to on page 105 and which may be fitted at either the eye or objective end of the body-tube, is employed. The lamp and bull's-eye have to be placed in fixed relation to this vertical illuminator, and it is important that once the illuminant is adjusted no more than a slight movement, such as would be imparted by the fine adjustment, should take place; this renders obvious the utility of the rack-work for raising and lowering the whole of the mechanical stage. Other microscopes for the same

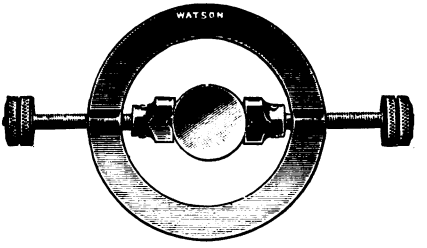

FIG. 12.—HOLDER FOR METALLURGICAL WORK.

specific purposes are made by C. Reichert, of Vienna, and Queen and Co., of Philadelphia.

The necessity for a special microscope for metallurgical work has, to a certain extent, been obviated by the introduction of a carrier, which can be constructed to fit any ordinary microscope stage. It is shown in Fig. 12. The subject for examination is held between two jaws, which can be made to approach or recede from each other by means of screws, to which they are attached, the block of metal under examination is gripped between the jaws, and can be set at any desired angle. This is particularly useful where large surfaces are required to be gone over or where the face of a cylindrical piece of metal is to be inspected, the cylindrical portion itself being suspended through the aperture in the stage. The addition of the vertical

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illuminator, referred to previously, with this fitting renders an ordinary microscope thoroughly serviceable for metallurgical purposes, but it would not, of course, have the vertical adjustment to the stage itself.

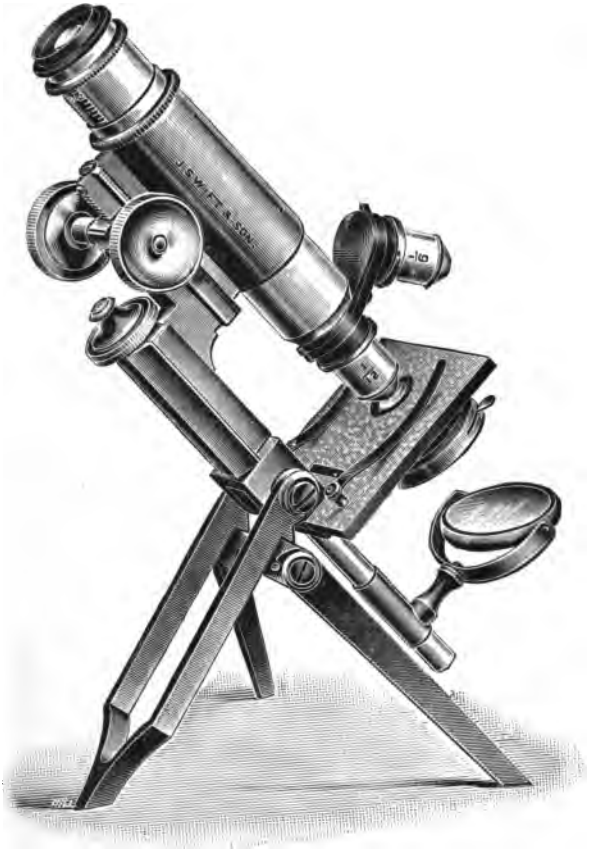

FIG. 13.—TRAVELLERS' MICROSCOPE.
By J. Swift and Son.

PORTABLE OR TRAVELLERS' MICROSCOPES.

A large variety of these are made, every maker having some pattern possessing especial merit. Among these may

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be mentioned very excellent models by Swift and Son, R. and J. Beck, Leitz, and Watson and Sons, the last-named offering to construct a body-tube of a size to carry eye-pieces of any desired gauge, so as to save duplication of apparatus. The instrument illustrated in Fig. 13, by Swift, is of ingenious and exceedingly compact and efficient design.

PETROLOGICAL MICROSCOPES.

A variety of microscopes are made for this purpose only, but those who may wish to examine rocks and crystals and to use their instrument for ordinary work as well will find it advisable to have a concentric rotating stage with the periphery divided and to read by verniers, a polarizer having a divided rotating circle, and immediately above it and fitting in the sub-stage with it a condenser of large aperture. The analyzer may be arranged to fit together with divided circles over the eyepiece of the instrument. Cross-webs can be fixed to the diaphragm of the eyepiece, and a calcspar plate can be fitted immediately beneath the analyzer prism in its carrier over the eyepiece. In the regular petrological microscopes these arrangements are already made, and in such, an extra analyzer is usually mounted in a box in the body of the microscope in such a manner that it can be pushed out of the field of view when desired. For petrological study exclusively, the instrument designed by Mr. Allan B. Dick and manufactured by Swift and Son, is usually conceded to be the most efficient pattern that is made. The special feature of this is that the stage is fixed, and instead of rotating the object, the polarizing and analyzer prisms with the eyepiece, are made to revolve together. Much of the time which ordinarily is occupied in effecting the exact centring of the stage to insure concentricity of revolution is thereby obviated.

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FIG. 14.—PETROLOGICAL MICROSCOPE.
By Swift and Son ; designed by Mr. Allan P. Dick.

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MICROSCOPES FOR PREPARING AND MOUNTING.

A plain, substantial microscope-stand, which the user would have no compunction in soiling, is of inestimable value in the preparing and mounting of micro-slides, and a suitable one can be obtained for such a small sum that it is a pity that instruments designed for work of a higher grade should be employed for the purpose. We figure on page 43 an illustration of the type of microscope that will be found very serviceable for this purpose. When we mention that the cost of this is only 20s., that it is provided with rack-work and pinion, and that it has the standard screw for objectives, and receives eyepieces of Continental diameter, it will probably need no further recommendation.

THE SELECTION OF A MICROSCOPE.

It will be well to summarize the conclusions arrived at in the consideration that has been given to the individual parts of the microscope in the foregoing pages.

In the interests of the advancement of microscopy as a science, the best and most suitable means should be commended, and nothing can be said to encourage the perpetuation of such instruments as do not embody the accuracy of adjustment or convenience of design which the modern worker with his beautifully perfect optical accessories actually needs in order to derive all the benefit that his lenses are capable of yielding.

In perusing the catalogues of the Continental makers it is impossible to find amongst them a single instrument which combines all those refinements which the English manufacturer has with such excellent judgment devised and fitted in his. We have had occasion to refer previously to the fact that the Continental sub-stages are provided with no centring screws, the stages, except in two cases,* have

* Photographic microscopes by C. Zeiss and C. Reichert.

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FIG. 15.—STAND VI., BY LEITZ.
A very inexpensive microscope suitable for preparing and mounting specimens.

no mechanical screws, and the fine adjustments, with rare exceptions, which have been duly acknowledged, are far too rapid in their action.

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It is asserted by users of Continental microscopes, whose name is legion, that the British microscope exceeds the needs of the laboratory worker; the only response to such a criticism from the expert is that there is a want of appreciation and education in matters microscopical in the laboratory. It is impossible to disregard the modern spirit which demands excessive rapidity of work at the cost of excellence and accuracy, and it is not too much to say that the man who examines structures with a good $\frac{1}{12}$-inch oil immersion objective and an Abbe illuminator has limited his knowledge to an extent which would cause him great surprise if he had but the opportunity of seeing the same subject properly illuminated with a sub-stage condenser having a suitable ratio of aperture to that of the objective and the microscope properly manipulated. The defects of the Continental microscope are in a large measure diminished because of this very restricted cone of illumination which is yielded by the Abbe illuminator.

The modern $\frac{1}{12}$-inch objective having a numerical aperture of 1·25 to 1·3 will bear a solid cone of illumination of 0·9, and when it is stated that the Abbe illuminator regularly provided, not only by Continental opticians, but also by English houses, only yields an aplanatic cone, under the most favourable circumstances, of 0·5, it will become only too apparent how utterly restricted the laboratory worker becomes in his work when outfitted with the apparatus which the Continental optician provides. When once the Abbe illuminator is abandoned and a suitable well-corrected condenser is substituted for it (for be it remembered the Abbe illuminator is not even achromatized) it will become necessary to improve the construction of the Continental stand. Centring screws must be perforce provided to the sub-stage ring; fine adjustments to the sub-stage, though not indispensable, will soon be found desirable, and the inventive faculty will quickly be brought into play for the provision of a slower acting fine adjustment. As matters stand to-day it is impossible to do other than advise that an English

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microscope by one of the well-known makers should be selected, and the following would be the order of preference for the various mechanical fittings:

1. Coarse adjustment by rack-work.
2. Fine adjustment.
3. Compound sub-stage with screws to centre.
4. Mechanical movements for the stage.
5. Mechanical draw-tube.
6. Fine adjustment to sub-stage.
7. Concentric rotation to the stage.
8. Divided scales, as may be found necessary.
9. Other mechanical fittings, such as centring screws and rack-work to the rotation of the stage, rack-work rotation to sub-stage, etc.

In amplification of the above we would remark that where questions of economy prevail the sub-stage may be replaced with an under-fitting having centring screws and the mechanical stage with a sliding bar.

Many microscopes are made in plain form as a foundation on which as a super-structure many of the mechanical fittings can be subsequently mounted; consideration might with propriety be given by a beginner to such instruments.

TESTING A MICROSCOPE.

The following are some points to be specially examined when purchasing a microscope: The motions should be perfectly smooth, with no lumpy feeling, and there should be no backlash. This latter can be detected best by holding gently the part that is actuated by the pinion, and then attempting to rotate the pinion. If the pinion rotates at all, or a movement of it can be detected without a corresponding motion on the movable part, there is backlash. Then, there should be no shake in any of the fittings. In a badly-constructed microscope, even when the fittings are in their most advantageous position, by holding them and shaking them slightly, a movement in the slides

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can be detected. The body should be racked up a considerable distance to see whether any rock or shake beyond that of the tension on the bearings can be detected. An instrument sound in construction should exhibit none whatever. The stage should be treated in the same way.

The next point is to ascertain that the body and limb are perpendicular to the stage; this should be tested with a metal square, such as is generally used by brass workers, engineers, and others, and can be purchased at a tool-shop. If there be any inaccuracy in this respect it is important that it be discovered before the microscope is used, otherwise no objective can possibly work at its best. Reference

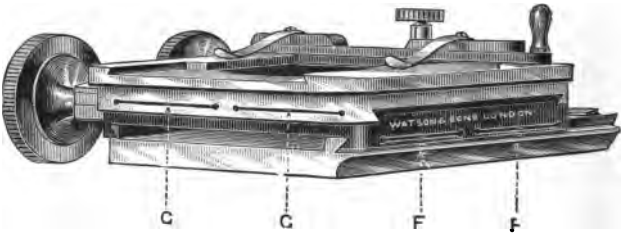

FIG. 16.—MECHANICAL STAGE SHOWING THE 'SPRUNG' FITTINGS WITH ADJUSTING SCREWS.

has already been made to the necessity for seeing that there is no lateral movement in the fine adjustment and that the sub-stage centres accurately. Beyond this it is well to put a square between the upper surface of the sub-stage and the under-side of the stage to ascertain that they are parallel and that the sub-stage has been mounted with accuracy.

It is also of importance that each fitting in which movements are effected should be provided with slots and screws, by which the effects of wear and tear can be taken up. This is known as 'springing' the fittings, and the system is shown very clearly in Fig. 16. Screws are placed at F and G, which, on being tightened, produce a compression of the fitting in which the slots are made, and wear can quickly be adjusted for.

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A good idea of the comparative quality and finish can often be obtained by examining some hidden or unnoticed part, and observing whether the same care in finishing has been exercised there as in parts that are seen. For instance, if some microscopes be examined underneath the foot, they will be found left in the rough as cast, and merely blackened over; while another instrument will be found carefully finished in that part. It would not necessarily follow that the former was a bad instrument, but it would often be found, if taken to pieces, that there was not a careful fitting in working parts that did not catch the eye, and there would be a probability of its not proving so durable as the better-finished instrument.

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CHAPTER II.

OPTICAL CONSTRUCTION.

Preliminary Note.

In the former part of this book we have dealt exclusively with the stand, or mechanical means of employing the optical system and accessories; and important as it is that those details shall be very efficient, it is, if anything, still more so that the eyepieces, objectives, and illuminating apparatus shall be of the most perfect description, properly adapted and intelligently employed, for on the optical combinations depend the results that are to be obtained with the stand; and although care and trouble may enable a person to use a bad stand, no good stand can ever compensate in any way for bad objectives. It requires constant practice and a long apprenticeship to learn to use the microscope to the utmost advantage. Every special subject of examination calls for special manipulative treatment if it is to be correctly understood and appreciated. Experience alone can guide in obtaining the best result under varied circumstances, and that experience must be based on a knowledge and understanding of correct methods in working.

Definitions.

Some of the following terms will be made use of in this book, and are constantly met with in literature on microscopical subjects; a brief explanation of them may therefore prove of service.

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Achromatic Correction.—Owing to the relatively greater dispersive power of flint-glass (containing lead or other heavy metals) as compared with crown-glass, it is possible to produce a combination of a convex lens of crown-glass with a concave lens of flint, which collects rays like a simple convex lens, but which unites two different colours in the *same* focal point, thus in a great measure correcting the chromatic aberration.

Aplanatism.—A freedom from spherical aberration (see below).

Apochromatic Correction.—The highest attainable correction of microscope objectives, comprising the correction of spherical aberration for all colours, and the union of *three* different colours in one focus, thereby eliminating the secondary spectrum.

Chromatic Aberration. — White light is the composite effect of a continuous range of colours, passing from red, through yellow, green, and blue to violet (see Spectrum). All transparent media have different refractive indices for these different colours, and as a consequence after their passage through a simple lens the rays do not unite at one focal point. The red rays, being the least refrangible and bending to the smallest extent, unite at the farthest distance from the lens; the orange and green rays unite at points closer to the lens; while the violet rays come to a focus at a point nearest to the lens. The confusion of different coloured images resulting from this dispersion is termed 'chromatic aberration.'

Chromatic Over-correction. — A term used when a lens brings yellow or even orange rays to the shortest focus and best correction.

Chromatic Under-correction.—A term applied to a lens when rays towards the blue end of the spectrum are best corrected. Thus a photographic lens is *visually* under-corrected.

Diaphragm.—This is generally understood in optical instruments to be a circular opening in a plate that is

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used to cut off the marginal portions of a beam of light, and in this sense is referred to in this book. The diaphragm is often improperly called a *stop*.

Diffraction Spectra.—If we look through a finely-ruled grating at a gas or candle flame, we shall see a large number of images of that flame having the colours of the spectrum. This effect is due to diffraction. In the microscope, objects having fine and regularly spaced markings diffract the light in a similar manner, the resulting diffraction spectra being plainly visible at the back of the objective. According to the Abbe theory of microscopic vision these diffraction spectra determine the character of the image seen, the latter becoming less like the real structure when the number of diffraction spectra admitted by the objective is reduced, a faithful representation of the object being obtainable only when all diffracted light of sensible brightness is admitted. A further note on this interesting subject by Dr. G. Johnstone Stoney, F.R.S., will be found in an appendix on page 124.

Female and Male Screws.—The former is a threaded fitting which receives a screw, and the latter a screw which goes into the female fitting. In the case of a bolt and nut the former would have the male, and the latter the female screws.

N.A. — Abbreviation for numerical aperture. See page 60.

Negative Eyepiece.—This is an eyepiece for examining an image formed at the diaphragm set between the two component plano-convex lenses. The Huyghenian is the best-known form of negative eyepiece.

O.I.—Abbreviation for optical index. See page 64.

Positive Eyepiece.—This is an eyepiece for examining an image situated beyond the field lens. It can consequently be used as a magnifying-glass, etc.

Refractive Index.—When a ray of light passes obliquely from one medium into another of different density, the path of that ray is bent or altered in its course. According to

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the law of refraction, there is a constant ratio for any given two media between the sine of the angle of incidence (being the angle included between the incident ray in the first medium and the perpendicular) and the sine of the angle of refraction, or of the angle included between the ray after refraction and the same perpendicular. The numerical value of this ratio for a ray passing from air into a medium is called the refractive index of the medium.

Secondary Spectrum.—In an achromatic lens the chromatic aberration is corrected for the brightest (yellow or green) rays of the spectrum, and the pronounced colour shown by uncorrected lenses is in consequence removed. A stricter examination, however, shows that rays of a different colour are not brought to the same focus, for owing to the fact that flint-glass, as compared with crown-glass, disperses the more refrangible rays relatively too much, and the least refrangible relatively too little, a peculiar *secondary* spectrum results from the achromatic combination, the rays corresponding to the brightest apple-green part of the ordinary spectrum being very closely united and focused nearest the combination, whilst the other colours focus at increasing distances *in pairs*, yellow being united with dark green, orange with blue, red with indigo. The composite effect of these colours is best seen with oblique light, causing dark objects to have apple-green borders on one side and purple ones on the other.

Semi-apochromatic Correction.—In achromatic microscope objectives of the older type, chromatic defects that are worse than the secondary spectrum are caused by spherical aberration of the coloured rays, the spherical aberration being corrected for the brightest part of the spectrum only. Objectives made entirely of glass, and therefore showing the secondary spectrum, are called semi-apochromatic when the spherical aberration is corrected practically for all colours.

Spectrum.—A band of colours produced by the splitting up of white light by means of a prism. The order of the colours is: red, orange, yellow, green, blue, indigo, violet.

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Spherical Aberration.—Rays of light passing through the marginal portion of a lens come to a focus nearer to the lens itself than those rays which pass through the centre of the lens, and the interval between the focal points of rays which pass through the marginal and the central parts of that lens is the spherical aberration. In compound lenses this spherical aberration can be corrected for one or more special rays, and a lens so corrected is called aplanatic. It is only truly aplanatic for the particular rays for which it has been accurately corrected.

Spherical Over-correction is present when a lens limits the marginal rays at a greater distance than the central rays. Spherical over-correction is indicated when the marginal rays focus closer to the lens than the central ones.

Spherical Zones.—In objectives of considerable aperture the intermediate rays may show decided spherical aberration, although the central and marginal rays are united. This defect is meant when spherical zones are spoken of. The degree to which spherical zones are corrected determines chiefly how large a cone of illumination, and how deep an eyepiece an objective will bear before 'breaking down.' A high degree of correction for spherical aberration and spherical zone must accompany the reduction of chromatic defects before terms such as 'semi-apochromatic,' and especially 'apochromatic,' can be applied to a lens.

Stop.—In an optical instrument this is a means of obstructing the passage of the central portion of a beam of light.

MAGNIFYING POWER.

It is often supposed by the novice that magnification is dependent on the size of the instrument, and a large one is frequently described as a very powerful one. This is quite an error. Given suitable eyepieces and objectives, the same magnification may be obtained on a small microscope as on a large one. It is entirely dependent on the two optical parts,

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the objective, and the eyepiece or ocular. Under the head of 'Objectives' in the makers' catalogues it will be noticed that the powers are expressed as 1-inch, $\frac{1}{4}$-inch, $\frac{1}{8}$-inch, etc. The figures do not indicate the distance at which the lenses focus on the object, but are intended to approximately convey the equivalent focus, and thereby the actual magnifying power of the objective. To understand this description, imagine an objective to be placed so as to form an image of an object at 10 inches from its back lens.* Then, an objective which, when so placed, formed an image on the screen which was ten times (diameters) the real size of the object would be described as a 1-inch objective; one which formed an image twenty times the size of the object would be called a $\frac{1}{2}$-inch objective, and in general the result given, when the magnification of the image formed on the screen is divided into ten, is what is spoken of as the focal length of the objective; also the equivalent focus of an objective divided into ten gives its magnifying power—thus a 2-inch should magnify 5, a $\frac{1}{4}$-inch 40, and a $\frac{1}{8}$-inch 80, diameters. This is termed the initial magnifying power of an objective.

The foci of German objectives are usually expressed in millimetres, 250 millimetres (about $9\frac{13}{16}$ inches) being taken as the normal vision distance, and the focal length of the objective divided into 250 gives the initial magnifying power.* Thus a 3-millimetre objective should have an initial power of $83\frac{1}{3}$, and a 4-millimetre of $62\frac{1}{2}$, diameters, and so on.

The image formed by the objective is again magnified by the eyepiece. Unfortunately, the latter is rarely marked with its magnifying power, the general rule being to call the different powers by the letters A, B, C, D, etc., or 1, 2,

* The above plan will be sufficiently accurate for experimental purposes, but, strictly speaking, it is the equivalent focus of the objective which determines its magnifying power, and in order to obtain exact results the measurements should be taken from the upper focal plane of the objective. The optical tube-length should also be reckoned in like manner, and this may generally be assumed to be from $\frac{1}{2}$ inch to 1 inch longer than the mechanical tube-length or the length of the body of the microscope.

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3, 4, etc. This is not very intelligible, and it would be far better either to express their equivalent focus, as in the case of objectives, or to have the magnifying power in diameters marked on the cap. We will take it that the 'A' eyepiece yields a magnification of 5 diameters. When this, therefore, is used in conjunction with the 1-inch objective on a 10-inch tube-length, which according to the rules previously given would produce a magnification of 10 diameters, the resultant combined power is 50—that is, the powers of the objective and eyepiece multiplied together. The method of estimating the power with short tube-lengths is referred to on page 69.

OBJECTIVES.

For our purpose we shall divide the subject of objectives into two classes—(1) the apochromatic, and (2) the achromatic. The immersion objective which may belong to either of these two classes is referred to separately. As in our remarks on objectives we shall constantly use the two terms, we will describe their reference.

Apochromatic Objectives.—The introduction of these objectives by the firm of Carl Zeiss, of Jena, Germany, placed the science of microscopic optics on a far higher level than had hitherto been attained, and as a result many of the traditional modes of working have been altered. Greater precision has been necessitated in the microscope-stand, and the provision of sub-stage condensers of corresponding optical quality to the objectives has been essential. Professor Abbe and Dr. Schott were granted a subsidy by the Prussian Government with a view to the promotion of optical research, and aided by this they were able to produce several varieties of new optical glass. The employment of these new glasses in conjunction with fluorite, based upon the careful and elaborate calculations of Professor Abbe, resulted in the production of apochromatic objectives. In these lenses, aberrations which were inherent in the older systems were eliminated or minimized—that

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is to say, the secondary spectrum was practically removed, and spherical aberration was very perfectly corrected for all colours. The objective, therefore, produced, to all intents and purposes, a colourless image. Higher apertures were obtainable, and in consequence of the improved corrections, accompanied by greater brilliance of the field, the use of eyepieces of high power was rendered permissible and advantageous.

The new kinds of glass were placed at the disposal of opticians throughout the world, and apochromatic objectives have been since manufactured by other firms, and notably by Messrs. Powell and Lealand, of London, whose productions compare favourably with the best of the originators' lenses. The apochromatic objectives by Zeiss have their equivalent focus engraved in millimetres, and it is becoming usual for the same method to be applied to other objectives also. The initial magnifying power of such lenses is ascertained by dividing the equivalent focus in millimetres into 250. Thus, a lens with an equivalent focus of 2·5 millimetres would have an initial magnifying power of 100 diameters.

Special eyepieces, termed 'compensating oculars,' are necessary when using the apochromatic objectives. They will be found described on page 79.

Achromatic Objectives.—All objectives that are not actually comprised in the apochromatic category—that is, in which the secondary spectrum is not eliminated—are included under this heading. So far as the principal opticians are concerned, it comprehends a better class of objectives than it did at the period when apochromatic lenses were introduced. By the use of the new optical glasses previously referred to, and in consequence of keen competition amongst manufacturers, many achromatic objectives, tending towards apochromatism, have been made. Several of these are so well corrected that in some instances they vie with the apochromatics in performance.

The class has consequently arisen which has been

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referred to under the generic term of *semi-apochromatics*. Many of these lenses are made in such perfection as to be superior even in some features to the real apochromatics. Some of the lenses in Watson and Sons' new series of 'Holoscopic' objectives, which require to be used with over-corrected eyepieces of the compensating type, are especially free from spherical aberration. Messrs. Swift and Son, of London, in their series of pan-aplanatic objectives, produce beautiful results; also C. Reichert, of Vienna, and Leitz, of Wetzlar, make objectives that are worthy of special consideration. Beyond these there are most excellent series of lenses made by all the microscope manufacturers which meet the requirements of the ordinary amateur in a most efficient manner—in fact, the general quality of such objectives is superior to that which obtained in the so-called best lenses of a few years ago.

Achromatic *versus* Apochromatic Objectives.—In view of the foregoing facts, it will be well to consider which series of objectives should be selected for specific work. It has to be remembered that apochromatic objectives are very expensive, and, generally speaking, are beyond the reach of the ordinary amateur, who usually takes up microscopy without special scientific aims, and excepting to a trained critical eye they would not be found to possess the extraordinary merit that is claimed.

The question naturally occurs, Is it worth while to incur the great cost which is involved in the purchase of apochromatic objectives? No decided opinion can be given without a full knowledge of the scope of the work which is to be undertaken, and as from the nature of things at its inception it is impossible to tell the extent to which research may be carried, the difficulty of giving advice is increased. Generally speaking, it may be stated definitely that for the ordinary work of the amateur, the so-called students' series of lenses will be found to give all the pleasure and satisfaction that are to be derived from the examination of Nature's small things, without attempt-

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ing to obtain apparently impossible results or to detect structure not hitherto discovered. The man who definitely equips himself for original research cannot afford to have less than the very best means which modern optical skill can afford him, and from the point of view of actual supremacy the apochromatics must then be chosen; but he would be limiting his possibilities in practically no degree whatever by having lenses carefully selected from those previously referred to under the title of semi-apochromatics.

It should be remembered that the reduction of spherical zones enables a higher power of eyepiece to be employed with an objective than would otherwise be possible, and it is due to this quality that the aprochromatic objectives have been especially valuable.

A series of compensating eyepieces is specially designed to work with them, having powers varying from 2 to 27 diameters. Supposing, therefore, we were working with a ¼-inch apochromat having an initial power of 40 diameters, with a ten-inch tube-length, we could by means of the searcher eyepiece (× 2) obtain a magnification of 80 diameters, and by using intermediate powers of eyepieces up to the × 27, produce any magnification that might be desired from 80 to 1,080 diameters (¼-inch initial power of 40 × 27 eyepiece power = 1,080).

Further, these special eyepieces are all designed to work in the same focal plane at the tube-length for which the eyepieces and objectives are designed, with the result that practically very little re-focusing is necessary on the exchange of an eyepiece during an observation. By this means the magnification with a low power objective having a long working distance and a fairly high N.A. for its power, as possessed by all of the apochromats, of Messrs. Zeiss' manufacture, can be gradually increased, and the advantage gained is one for which many microscopists sighed before the days of apochromats—namely, a wide range of magnifying power and great working distance. For many classes of work this convenience is very great.

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Some of the best of the achromatic objectives, to which reference has already been made, will stand as high an eyepiece power as the apochromats, but generally they do not advantageously bear anything higher than, say up to 10 or 12 diameters, and usually not in the perfect manner that the apochromats do. The Huyghenian eyepieces that are used with the achromatics are very rarely designed to work one after the other in the same focal plane, with the result that it is necessary to refocus every time the eyepieces are exchanged, and the higher the power of the eyepiece that is employed the closer will the objective work to the object. The special convenience derivable with the apochromats is, therefore, practically non-existent with the achromatic objectives.

Magnifying power, however, is not the only feature to be considered with regard to an objective; there must be a power of delineating fine detail. This latter quality is dependent on the numerical aperture of the objective, referred to on page 60.

Immersion Objectives.—In using these objectives a film of a specified fluid is interposed between the front lens of the objective and the cover-glass of the object under examination, so that continuity is established between them. There are two media that are in regular use, viz., water and cedar-wood oil. Others, including glycerine and mono-bromide of naphthalin, are, however, occasionally employed. It may be taken that when a lens is referred to as a 'homogeneous or immersion' objective, that cedar-wood oil, or a mixture, of which that oil is the principal ingredient, known as immersion oil, is the correct medium for using with that objective. The refractive index of cedar-wood oil is about 1·52, and practically the same as crown-glass, consequently, when it is used for immersion purposes it has the effect of rendering the cover-glass part of the objective.

The question naturally arises, What advantage is gained by the use of an immersion medium? In reply, it may be briefly stated that the resolving power of the objective,

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the brilliance of the image, and the working distance, are all increased.

It is a well-known law that rays passing from a rarer to a denser medium are refracted towards the perpendicular, and *vice versâ*. If, therefore, an object be examined with a dry objective, it is obvious that certain rays of light emerging from the denser crown cover-glass into the rarer medium, air, are refracted so far from the perpendicular as to fail to assist in forming the image. By placing a medium between the cover-glass and the objective, these rays are utilized, owing to the influence of the dense medium, oil. The refractive index of air is 1·0, that of water 1·33, while that of cedar-wood oil is 1·52. It will be seen from this, that the utility of the oil must be very appreciable, in fact, an oil immersion lens receiving light at 82° and a water immersion lens receiving light at 96° admit the same rays as a dry lens of 180°, and, therefore, divide as many lines to the inch as the maximum number possible with a dry lens. If immersion lenses have greater apertures than the above-named they will divide finer markings than any dry lens, and they can be theoretically carried to oil and water angles respectively of 180°.

There is another feature of advantage gained by the use of an oil immersion lens. The refraction caused by the influence of the cover-glass thickness referred to on page 65 does not take place, on account of the continuity established between the objective and the cover-glass by the immersion oil. There is, therefore, no necessity for such objectives to be provided with a correction collar for variations in thickness of cover-glass; a slight correction of the same kind has, however, sometimes to be made on account of the distance which the object may be beyond the cover-glass when the mounting medium has not the same refractive index as the cover-glass. This can be efficiently effected by either extending or shortening the body-length. Water immersion objectives do not yield so high an aperture as the oil immersions, and as the

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immersion medium is not of the same density as the cover-glass a correction collar is essential, but there are subjects with which oil could not be suitably used, and in such cases the water immersion lenses have to be chosen.

Mono-bromide of naphthalin is, at present, only used with one form of objective, a $\frac{1}{10}$-inch, by Carl Zeiss, of Jena, having a numerical aperture of 1·63. The refractive index of this medium is 1·657, and special flint cover-glasses of the same density have to be employed with it. This restriction, together with its high price—£40—has prevented its being largely used. Those who have had an opportunity of working with one have spoken in high terms of the beautiful effects it yields.

It must be borne in mind that objectives that are intended to be used immersed are specially corrected for the specific medium to be employed. Ordinary lenses intended for use dry cannot be advantageously worked immersed.

APERTURES OF OBJECTIVES—ANGULAR AND NUMERICAL.

For reasons which will be stated hereafter, it will be seen that on the aperture possessed by an objective depends the fineness of detail that it is capable of delineating—that is, the number of lines per inch that it will separate.

Angular Aperture.—Before the introduction of immersion objectives the ability of an objective to resolve fine structure was known to be dependent on the angle formed by the extreme rays issuing from the object that could be received by the objective. This, which was called the angular aperture of a lens, is, in other words, the angle of the cone which envelops the pencil of light that is received by the objective from a point on the object.

As we have stated in the description of immersion lenses, an oil immersion lens receiving light at 82°, and a water immersion, receiving light at 96°, would each divide as many lines to the inch as a dry lens having the limiting

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aperture of 180° (which in practice can never be quite reached), and as the immersion lenses can theoretically be carried to oil and water angles respectively of 180°, it is obvious that in order to express the efficiency of such objectives a notation must be employed which takes cognizance of the medium which surrounds the front of the objective, and the result it has in the formation of the image. This is achieved by means of the system termed NUMERICAL APERTURE, which was introduced by Professor Abbe. This expresses the efficiency of an objective to allow pencils of light to pass so as to include them in the light forming the image. Numerical aperture is expressed in the formula *n sin u*. *n* signifies the index of refraction of the medium by which the objective front is enveloped, and *u* equals half the angle of aperture. Therefore, by multiplying the sine of the semi-angle of aperture by the refractive index of the medium in which that angle has been measured the numerical aperture (*n sin u*) is obtained.

It follows from this that the greatest value which the numerical aperture can have in the case of dry lenses is unity, corresponding to an angular aperture of 180°.

If we are aware of the numerical aperture of an objective we can readily ascertain the number of lines per inch or millimetre which it is capable of dividing, or, in other words, its extreme power of resolution. The formula is—twice the numerical aperture, multiplied by the wave-frequency* of the light used, equals the extreme number of markings per inch or millimetre, according as the calculation may be made, that the lens will resolve.† Conversely, if the extreme limit of resolving power be known, the number of lines per inch or millimetre that it will separate, divided by

* The wave-frequency is the number of waves contained in an inch or millimetre, according to which measure is used.

† The mean wave-length of white light is 0·5269 μ (= 48,200 to an inch). Taking the numerical aperture of an objective as 1·0 N.A., and for this purpose doubling it, we find that with the aperture of 1·0 N.A. 96,400 lines (about) per inch can be resolved with white light (48,200 × double the numerical aperture = 2, produces 96,400).

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the wave-frequency of light used, equals twice the numerical aperture.

These calculations are based on the assumption that annular or some other form of oblique illumination is used. With a solid cone of illumination equal to the numerical aperture of the objective no fine detail is visible; it becomes blurred, and, in practice, when using solid cones of illumination, it is usual to make them fill $\frac{3}{4}$ only of the back lens of the objective. This will be found treated on page 97, in connection with condensers.

Under these conditions the number of lines per inch that will be resolved by the objective will be ascertained by multiplying the wave-frequency of the light used by $\frac{3}{2}$, and then multiplying the product by the numerical aperture.*

THE APERTOMETER.—To enable the numerical apertures of objectives to be taken without a calculation, Professor Abbe devised the apertometer. It consists of an almost semi-circular plate of glass, having the diametrical edge ground to an angle of 45°, while the circumference is a polished cylindrical surface. It is shown in Fig. 17.

The centre of the semi-circle is marked by a silvered disc, *a*, having a very small central aperture, and on the upper surface on the periphery it is provided with a scale of divisions, indicating both angular and numerical apertures. The manner of using the apertometer is as follows: The microscope is placed in a vertical position, and the apertometer is laid on the stage, with the diametrical edge towards the limb of the instrument. The objective that it is desired to take the aperture of is then screwed on, and the objective focused on the plain centre of the silvered disc. It is well now to fix the apertometer to the stage, either by springs or an elastic band, to prevent its moving. The two pointers, *b*, are then set on the edge of the circle to read zero. The draw-tube and eyepiece with which the silvered disc has been set are removed, and at the lower end of the draw-tube a special objective of low

* E. M. Nelson, *Journal of the Royal Microscopical Society*, 1893, p. 15.

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power, that is supplied with the apparatus, is screwed into the universal thread, which should be there fitted in all microscopes of high-class.

The cylindrical edge of the apertometer is then illuminated in front and at the sides by means of bull's-eye condensers and lamps, or, if daylight is available, it will be easier to get uniform brilliance all over the field by placing the microscope on a table in front of a window, and using bull's-eye condensers to increase the light.

The draw-tube carrying the special objective and the eyepiece is then replaced in the body of the microscope and the image of the pointers *b* in Fig. 17 is brought sharply into

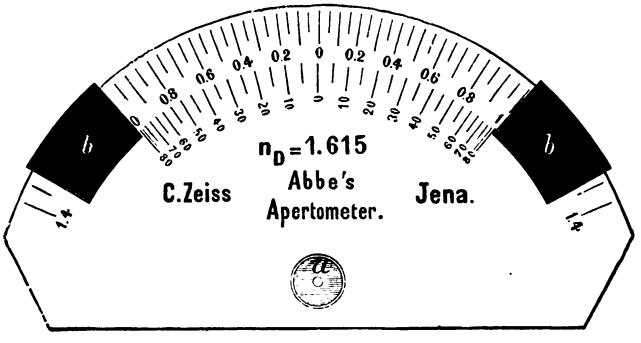

FIG. 17.—ABBE'S APERTOMETER.

focus in the centre of the field by slowly extending the draw-tube, being reflected by means of the prismatic diametrical edge of the glass plate. While looking through the microscope these pointers are then each moved separately, in opposite directions, round the outer edge of the apertometer, until they are set exactly on the extreme margins of the back lens. The reading is then taken by the divisions on the face of the apertometer against which the pointers have now arrived. In the case of an oil or water immersion lens, the medium must, of course, be placed in front of the object-glass during the examination.

There are several further matters of importance that it would be well to consider.

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Numerical Aperture and Power.—As we have before remarked, magnifying power is not the only quality necessary for the observation of minute structure. The power to delineate fine detail is still more dependent on the numerical aperture of the objective. It has been explained by Dr. Dallinger in 'Carpenter on the Microscope,' and will be evident from a consideration of the preceding remarks concerning numerical aperture, that two objectives—one of much greater magnifying power than the other, but both having only the same numerical aperture—will only divide the same amount of detail, the higher power only exhibiting it on a larger scale. That is, supposing with a $\frac{1}{4}$-inch objective of 0·90 N.A. certain structure were presented, and then a $\frac{1}{8}$-inch objective with just double the magnification, but with the same N.A., were afterwards used, there would be no further power of resolution in the $\frac{1}{8}$ than in the $\frac{1}{4}$. It might be possible to make an objective of very low power of sufficiently high aperture to divide very minute details, but this would be useless unless the objective would bear a sufficiently deep eyepiece to enable the human eye to see it. It therefore becomes necessary that a ratio of aperture to power should be established. Mr. Nelson has suggested that a standard, termed the 'optical index' (O.I.), should be adopted for this purpose, to indicate the numerical aperture that should be given to an objective, if it be intended that the eye should see in the image as fine detail as it could divide in a real object of the same size. It is ascertained by multiplying the numerical aperture of an objective by 1,000, and dividing by the initial magnifying power of the objective.* If a microscope is required to show all that keen eyes are able to appreciate, then 0·26 N.A. must be given to it for every 100 diameters of magnification. If we limit the power of the eyepiece of such a microscope to 10, then the objective must have 0·26 N.A. for each 10 diameters of *initial* magnifying

* E. M. Nelson, *Journal of the Royal Microscopical Society*, February, 1893.

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power. The optical index, therefore, of an objective which, with an eyepiece magnifying 10 diameters, will yield all that it is possible for a normal eye to appreciate, will be 26·0. In practice it is found possible to employ eyepieces giving higher magnifications than those mentioned in Mr. Nelson's rule, and these would of course enlarge the image.

Although large apertures are the pride of those whose ultimate ambition in matters microscopical seems to be bounded by the endeavour to resolve the markings upon diatomaceous frustules, it is doubtful whether for the ordinary amateur there is a real necessity for the extremely large apertures. Lenses having such, require great skill and care in manufacturing and adjusting, and are consequently expensive, and if the ordinary work of an amateur is proposed to be conducted, and not original scientific research, objectives of medium aperture will usually be found to meet his requirements thoroughly.

THE INFLUENCE OF THE COVER-GLASS.

As a rule, objectives are corrected for a specified thickness of cover-glass, which is placed over the object to protect it. These cover-glasses, however, vary considerably in thickness, and consequently by refraction disturb the corrections of the objectives. An objective which gives crisp definition when an object that has no cover-glass to it is being viewed, will not define so clearly if a thin one be applied, and the greater the thickness of the cover-glass the more will the image be deteriorated.

In other words, spherical aberration in the sense of under-correction is introduced when the cover-glass is thinner, and in the sense of over-correction when the cover-glass is thicker, than that for which the lens was adjusted. This spherical aberration arises from the refraction of the rays in the plane surfaces of the cover-glass and objective front respectively; it is negative for the cover-

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glass surface, and positive in the front lens plano, the latter preponderating owing to the greater diameter of the cone of rays from the object where it enters the front lens. With the correct thickness of cover-glass, the remaining under-correction is exactly balanced by an equal over-correction in the lenses of the objective, but a thin cover-glass produces insufficient over-correction, the diameter of the cone of rays being too small when it meets the surface of the cover-glass. A thick cover-glass produces the opposite effect—that is, the cone of rays is too large.

Low powers are not so sensitive to this influence as high ones. There are two means of correcting this. Dry objec-

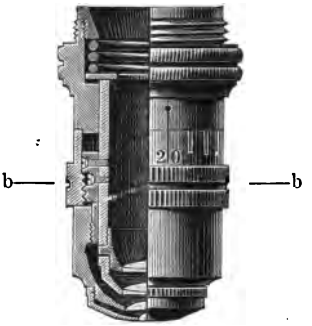

FIG. 18.—CORRECTION COLLAR (ZEISS).

tives having a large numerical aperture are often provided with what is termed 'a correction collar,' whereby the two back combinations of the objective are removed farther from, or brought closer to, the front lens or lenses. Fig. 18 shows the manner in which this is effected in one of Zeiss's lenses. bb is the correction ring, by turning which the distance between the upper lenses and the two lower lenses is varied. With such a correction collar a worker is undisturbed by thickness of cover-glass, because he has within certain limits the means at his disposal in the objective itself for correcting same. The use of this correction collar almost requires a personal demonstration, and to set it at the exact point that yields the best result

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is a matter of extreme delicacy, which can only be accurately done as the result of experience and with the aid of a critical eye. It is hardly to be recommended to students, because they will not usually afford the time and trouble necessary to get such perfect results; consequently there is a growing tendency, except in the apochromatic objectives, to have the lenses mounted in a rigid setting, corrected for a specific tube-length and thickness of cover-glass. With the fixed setting, if a different thickness of cover-glass be used than that for which the objective was designed, correction can be made by altering the tube-length of the microscope. This has the same effect as altering the distance between the lenses. Supposing we had an objective adjusted for a 6-inch tube, with a 'B' eyepiece, on a cover-glass 0·008 inch thick (this is about the average thickness adopted by opticians), and we wished to examine an object having another thickness of cover—say 0·005—we should at once notice that the performance was not so good, and in order to improve it we should have to make the body longer. This difference of cover-glass thickness, with a good $\frac{1}{8}$-inch objective, would necessitate a 10-inch body. On the other hand, if the cover were 0·01 inch thick, the length of the body would have to be diminished below that for which the objective had been designed, to obtain the best results—that is, for a thin cover the body-tube would have to be lengthened, while for a thick one it would have to be shortened, and the finer the quality of the objective the more sensitive would it be to cover-glass thickness. This system of correcting by draw-tube, however, has one drawback, and that is, that the power is varied in correcting, and, of course, the focus is altered. From the considerations here named, it will be found advantageous if the microscope be provided with a means of lengthening the body by draw-tubes to 12 inches, and on the other hand, when the draw-tubes are closed, of having the body shorter than the Continental length (6 inches). In order that the best adjustment may be made, it is essential that one of the

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draw-tubes be actuated by rack and pinion, and the convenience of this arrangement cannot be too strongly urged upon microscopists. Messrs. Baker, Beck, and Watson and Sons have adopted it in their large models, and it has evidently met with considerable appreciation, for it is now made in a cheaper form by the latter firm and Messrs. Swift and Sons for their students' instruments, and anyone with a limited purse, but who wished to do his work in the most precise manner, could have it in an inexpensive form of instrument.

DIRECTIONS FOR USING A CORRECTION COLLAR AND CORRECTING BY TUBE-LENGTH.

This may be accomplished in a systematic manner if it be borne in mind that the aim is to eliminate spherical aberration, which defect may be defined as a difference of focus between the central and marginal zones of an objective. Hence the correct tube-length or the best position of the correction collar has been found when some strongly-marked detail or outline of the object remains in exact focus under *any* change of illumination, say from a small to a large diaphragm opening beneath the condenser, or, better still, by changing the illumination from central to very oblique, these changes being made with great care, so as not to disturb the other adjustments.

The following process will be the safest and quickest: Start with the shortest tube-length, or when there is a correction collar, with the position corresponding to the thickest cover-glass; carefully focus some sharp outline with, say, a $\frac{1}{4}$ central cone, then change to a $\frac{3}{4}$ cone, or, better still, to very oblique light. Unless the object—owing to an exceptionally thick cover-glass, or a very badly adjusted lens—is beyond the range of your adjustments, you will find evidence of under-correction—that is, the lens will have to be brought closer to the object with the wide cone, or oblique light, than with central light.

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Gradually lengthen the tube, or turn the collar, repeating the above observation after each change, until all evidence of spherical aberration has disappeared; the instrument is then in correct adjustment within your own limits of vision.

It is advisable to start with the adjustment corresponding to the thickest cover, for the simple reason that this lessens the danger of running through the cover-glass and destroying the object, and possibly the front lens of the objective, when dealing with a lens of a short working distance.

The difference between an objective adapted to a 6-inch and that for a 10-inch tube is, that in the latter case the back combinations of the objective are brought closer to the front lenses; this gives a slightly increased aperture. The majority of cover-glasses that are purchased and a large number of those used over commercial objects are more than 0·008 inch thick; 0·008 inch is a medium thickness of cover-glass, but the tendency is to use thicker ones. It will be found a great advantage to buy only such objectives as are corrected for the 10-inch tube, and having the rack-work before referred to fitted to the microscope tube, sufficient latitude would still be allowed if a thinner cover-glass were met with; but it would often be found necessary to close the draw-tubes down to 6 or 7 inches, in order to get the best correction for the thick cover-glasses that are commonly used.

We may here clear up another question that occasionally arises. If a $\frac{1}{6}$-inch objective is corrected for a 6-inch tube-length, it does not give a magnification of 60 diameters at 6 inches. The powers of all objectives are calculated for a 10-inch tube-length, therefore the full total benefit is not obtained from an objective when used at 6 inches, but only six-tenths of it. Of course, with the lessened magnification at 6 inches a brighter field is produced, and a deeper power of eyepiece is found permissible. This is rather an important item in testing an objective, because an objective at 10 inches would be yielding about two-thirds more magnification than at 6 inches, and its

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powers would be much more severely tested than if employed at 6 inches.

It would be a great advantage to the microscopist if opticians would mark exactly the focal power and precise numerical aperture of their objectives upon them. In order that objectives may appear to have a large ratio of aperture to power, they are often put forward as possessing a considerably lower power than they actually have. For instance, a so-called 1-inch often turns out to be nearer $\frac{2}{3}$-inch, $\frac{1}{2}$-inch about $\frac{4}{10}$-inch, $\frac{1}{3}$-inch to have the power of $\frac{1}{6}$-inch, and $\frac{1}{12}$-inch in some instances to be $\frac{1}{14}$-inch. It has become such an acknowledged fact that the act of misrepresentation involved seems to be condoned. This is a state of things which should not be. Opticians must be aware of the misdescription and the immensity of trouble that is caused by it. We must, therefore, advise microscopists not to rely on the powers marked on their objectives, but to ascertain them for themselves, and the best way to do it is to project the image of a micrometer, without any eyepiece in the body-tube, on a screen 20 inches distant from the back lens of the objective. Measure with a foot rule the distance apart of the lines so projected, and supposing that each hundredth of an inch measured on the screen 1 inch, that would represent a magnification of 100 diameters; divide the distance used (20 inches) by the magnification found (100 diameters), and the result ($\frac{20}{100}$ or $\frac{1}{5}$-inch) is the equivalent focus or 'power' of the objective.

TESTING OBJECTIVES.

It is a somewhat difficult matter for the novice to decide for himself as to the quality of object-glasses. Such work needs experience, judgment, and a trained eye. The writer has met with people who have not been able to distinguish the difference in performance between an uncorrected single French lens and a first-class achromatic. This, of course,

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was due entirely to a lack of that perception of microscopical detail which can only be acquired by intimacy with objectives and their qualities. Especially is this true in lenses of the highest grade. We propose, therefore, to give a few hints which, if not of so much use in the initial stage, may be of aid at a later period.

Flatness of Field.—This is an important feature in objectives of low power, and the best method of testing for this quality is either by examining the ruled lines of a stage micrometer or the surface of a piece of ground glass. With the higher powers this feature is sadly neglected, especially in lenses of Continental make, it being advocated by one or two leading workers that it is better to get the utmost perfection of definition in one central point rather than that definition should be sacrificed for flatness of field. It would certainly be a great advantage to the microscopist if the two points could be combined in a more satisfactory manner than at present. English manufacturers in the high powers generally provide a flatter field than their Continental contemporaries.

Colour.—Dr. Carpenter's old test for achromatism—the examination of the cells in a thin section of deal—will give a very good idea of the colour corrections of objectives. For high powers, the markings on a frustule of the diatom 'Pleurosigma formosum' are an excellent test. With the apochromatic objectives these come out quite black and white, while with those of the achromatic series any outstanding colour is at once revealed. Another method is the mercury test adopted by opticians. A small globule of mercury is placed on a slip of ebonite, and a piece of whalebone or watch-spring is made to snap on it, causing the globule to split up into numerous particles of exceedingly minute size. These globules are then examined with the objective, and can be illuminated by means of a bare gas-jet, lamp, or daylight. Outstanding colour will be revealed by the globules.

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The Abbe Test-Plate.

The most satisfactory way of testing an objective that is at the disposal of him who would learn the whole inwardness of his lens is the Abbe test-plate. A considerable amount of experience will be required to use it advantageously, but it discloses at once, when employed by one who has learnt to appreciate its significance, any mechanical inaccuracies that may exist. Directions for using accompany each plate, but the worker will quickly mark out for himself a line which experience will show him is the best for quickly ascertaining whether the lens is accurately centred and the state of the corrections for spherical and chromatic aberrations. The test-plate itself consists of six

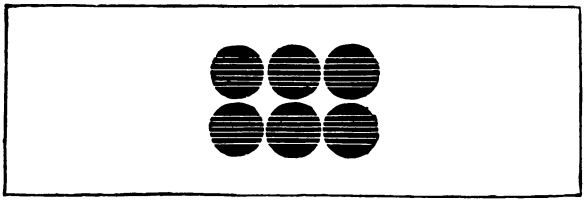

FIG. 19.

discs of cover-glass, all of different specified thicknesses and embracing such a range as objectives are likely to be corrected for.

On the under-surface, lines are ruled in a deposit of silver, and the covers are mounted on an ordinary 3 × 1 slip. The ruled lines are coarse, and can be separated with a low-power objective. The procedure adopted by the writer is as follows:

The tube-length should be that for which the objective is ostensibly corrected. An eyepiece of high power and a substage condenser, giving a solid cone equal to at least two-thirds the total aperture of the objective, are used.

In this connection it may be mentioned that it will be found advantageous to have an eyepiece which permits of some degree of over or under correction being obtained—

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such, for instance, as will be afforded by the Holoscopic eyepiece described on page 80. Those who have not such a convenience may unscrew the eye lens of the Huyghenian eyepiece and so secure some slight modification of correction. The object of this is to ascertain whether the lines may be rendered free from coloured edges or with the same colour on both edges all over the field. If in this preliminary step it be found that the definition is unsatisfactory, thicker and thinner cover-glasses should be tried; and in the event of failure to secure good definition in this way, and no reasonable alteration of tube-length will produce the desired effect, the objective may be safely rejected as bad.

It probably will be found that under one of the cover-glasses the lines will appear satisfactorily defined, in which case the centring may be examined.

DEFECTIVE CENTRING shows itself (*a*) by the impossibility of removing the coloured edges of the lines all over the field even when the eyepiece is adjusted as described above, the edge colouring being more apparent on one side of the field than the other; (*b*) by unequal definition of the two edges of the central lines, one edge appearing sharp or nearly so, while the other edge is seen double or foggy.

SPHERICAL ABERRATION.—The fact that the objective will bear high-power eyepieces on the test-plate in a satisfactory manner is in itself proof of good correction in this respect, but the following is a further excellent and convincing test:

Place a diaphragm beneath the condenser having an aperture that will cause the condenser to yield a cone of illumination equal to one-fourth the N.A. of the objective under examination, and while observing the lines change the position of this diaphragm from central to extremely oblique, the obliquity being in a plane at right angles to the direction of the lines. This is best performed by means of one of the mechanical condenser carriers, such as are provided in the Continental microscopes, one of which is figured in the microscope on page 12. If the lines remain

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sharp throughout, the corrections for spherical aberration are eminently satisfactory; but should a difference of focus occur, to avoid all chances of erroneous deduction the other discs should be examined to insure that the proper thickness of cover is being used; also the tube-length might be varied, and if after these precautions it is still found that there is a difference of focus over the intermediate position of the diaphragm, the existence of a spherical zone is at once demonstrated. This process enables the best tube-length and thickness of cover for the objective to be discovered with accuracy.

Chromatic Correction.—Tests for this should be made in the same manner as for the spherical correction. Under the same conditions an apochromatic should show practically no colour, or, at the most, barely distinguishable traces of tertiary tints. Semi-apochromatics, or lenses of fine correction, will show narrow bands of pale green (apple-green) on one side, and faint purple (or claret) on the other side, of each line, and the same colours or tints should appear whether the diaphragm be used centrally or obliquely, the width of the colour bands only changing; further, good definition should be yielded under all circumstances. Ordinary lenses will generally show the best colour correction for some intermediate zone. If they exhibit broad bands of primary colour—yellow or blue—with very oblique light, the definition will be found to be bad.

Curvature of Field.—There is one point concerning which some slight difficulty may arise in connection with the curvature of the field. Absence of flatness of field, which is inherent in the construction of all latter-day objectives, and particularly so in those of high power and large aperture, is not regarded as a fault, but coma in an objective at first sight gives the same appearance; but the difference is this: when the latter fault exists only the central line or those nearest to it can be focused sharply, those towards the margin remaining indistinct or ill-defined

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when re-focusing is attempted; in a well-corrected lens all of the lines will become sharp and distinct when the particular zone is adjusted for.

It will be found advantageous to confirm the observations on the test-plate by examinations of known test objects, and with practice the two together will soon enable reliable estimates to be formed of the quality of objectives.

Tests for Definition.—Use an eyepiece with the objective under examination that will give a total magnification in diameters equal to one thousand times the numerical aperture of the objective—that is, if a ½-inch objective, having a magnifying power of 20 diameters on the 10-inch tube, had a numerical aperture of ·45, an eyepiece, the magnification of which was 22½ diameters, would be necessary to give the required 450 diameters. If an objective bears this without serious breaking down its definition may be considered to be good. This test has the advantage of being based on a rational foundation, the ratio being the same as an eyepiece power of 50 to each inch of aperture in an ordinary telescope. This, again, is equal to what would be seen of an object if looked at through a pin-hole $\frac{1}{50}$-inch in diameter, beyond which the outlines of objects fail in clearness.

For objectives varying in power from 2 inches to ½ inch, nothing is better as a test than the proboscis of a blowfly. The spines in the central portion of the tongue should each show a well-defined point. For high-power objectives the internal markings of Triceratum and Pleurosigma angulatum, also the markings on the scales of Podura (Lepidocyrtus curvicollis), are the most suitable.

CHOICE OF OBJECTIVES.

The best objectives for a novice at starting would be 2-inch, 1-inch, and ⅛-inch. The 2-inch will be found extremely useful for large specimens, while the 1-inch, which is considered the working-glass of the average micro-

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scopist, will with a higher power—namely, the $\frac{1}{6}$-inch—show him some of the minuter detail which sooner or later he will wish to make himself acquainted with. If more object-glasses than these be required, we should recommend the $\frac{1}{2}$-inch as an intermediate between the 1-inch and the $\frac{1}{6}$-inch, and for a higher power a $\frac{1}{12}$-inch oil immersion objective should be added. It is well to buy only such low-power objectives as have double combinations. Some of the cheaper ones consist of two or three lenses balsamed together in one combination only; with these there is an insufficiency of aperture, and good definition and flatness of field cannot be obtained. All the best low-power lenses are constructed with two pairs or more of lenses set a little distance apart, and can be readily recognised. Of the apochromatic series of Zeiss our choice would be the 24, 12, 6, and 3 (1·4 N.A.) millimetre objectives if for the English tube-length, or the 16, 4, and 2 (1·4 N.A.) if for the Continental tube-length. The 12 and 3 millimetres are considered the finest of the series. Messrs. Powell and Lealand supply a $\frac{1}{12}$-inch apochromatic oil immersion objective of 1·4 N.A. which enjoys special favour, the price of which is only £10. Of achromatics that approach the apochromatics in performance the 8 millimetres by Reichert and his 4 millimetres (1·3 N.A.) homogeneous immersion are very fine, especially the latter. We have worked with both of these, and they have given most excellent results. The No. 6 and $\frac{1}{12}$-inch of the same maker are also extremely good. Leitz, of Wetzlar, is the maker of a very good series of achromatic objectives also, and his $\frac{1}{10}$-inch, £3 15s., and $\frac{1}{12}$-inch, £5, both for oil immersion, are excellent. As previously remarked, competition has caused all the makers to bring their objectives to a high level of perfection, and the novice will be quite safe in equipping himself with those of any of the leading English opticians. The productions of American opticians are but little known in England, yet they are said in many instances by competent judges to be of exceptional quality, Messrs. Bausch and Lomb and the

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Spencer and Smith Optical Company having high reputations for their lenses.

Of course, different requirements would necessitate the selection of special objectives, but a practical microscopist or any microscope manufacturer would be able to advise on the matter.

EYEPIECES.

The eyepiece commonly used with the microscope is what is termed the Huyghenian form, which generally consists of two plano-convex lenses placed at a distance apart about equal to half the sum of their foci, with a stop in the principal focus of the eye-lens. This will be found to meet all ordinary requirements of microscopical work with achromatic objectives. Eyepieces vary in power, and these powers are usually designated by the letters A, B, C, D, etc., A being the weakest power. On the Continent they are generally designated 1, 2, 3, 4, etc., while some firms express their power in focal units—for instance, an eyepiece having a power of 10 would be 1 inch. This last method, or that adopted by Zeiss for the compensating eyepieces, and several progressive English houses for their ordinary eyepieces, where the actual magnifying power is engraved on the cap of the eyepiece, is the only rational one. The letters A, B, C, etc., or Nos. 1, 2, 3, etc., convey no real idea of the magnifying powers of the eyepieces, because each maker has his own formula for each eyepiece, and there is no correspondence in the powers of one eyepiece marked 'D' by one maker, and that supplied by another. It is often remarked that the Continental objectives stand a stronger power of eyepiece than the English, and on this account a superiority has been claimed

FIG. 20.—HUYGHENIAN EYEPIECE.

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for them; but it should be borne in mind that English manufacturers give in many instances as deep a power of eyepiece as 20 or 25, whereas Continental manufacturers rarely supply them of greater magnifying power than 10 or 12 diameters. In the English series the variation in power between two consecutive eyepieces is generally greater than in the Continental series. A comparison, therefore, between the merits of an English object-glass tested with, say, a 'D' or No. 4 English eyepiece and a Continental object-glass of the same power tested with a No. 4 eyepiece of Continental make would not be fair, as the former, having a deeper power eyepiece on it, would be liable not to give such perfect results as the latter. And here we may mention that, although people very often buy deep-power eyepieces, it is advisable, with ordinary achromatic lenses, that no stronger power should be used than an eyepiece giving an initial power of 10 or 12 diameters. The best eyepiece for general purposes is the 'B.' This gives a convenient size of field, and is by far the most comfortable to work with of the whole series. Next, and in addition to this, we should recommend either the 'C' or the 'D.'

Microscopists having abnormal vision, and preferring to work without spectacles, should have an auxiliary cap made to fit over their eyepieces, carrying a lens of the power that corrects the error of vision. This is especially necessary where measuring has to be done or where the microscope is arranged for a second person's inspection.

At times it is desired to know whether an eyepiece can have its diaphragm enlarged so as to give a larger field. An easy method of ascertaining how much of the field lens is employed is to make a spot with ink near the margin on the convex side of the field lens, and on placing the eyepiece in the microscope, if the diaphragm has a sufficiently large aperture, the ink will be visible; if not, it may be enlarged until it appears. The diaphragm should not be so large as to admit of more than the edge of the field lens being visible.

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Compensating Eyepieces.

Under the description of 'Apochromatic Objectives' on page 57, reference is made to compensating eyepieces. These are specially designed to correct an outstanding colour defect (of the nature of under-correction) which is inherent in all high-power objectives, whether they be apochromatic or achromatic, on account of the peculiar construction of the front lens. For the sake of uniformity of eyepiece, Zeiss imparts the same colour effect to the lower-power lenses of the apochromatic series. The eyepieces, then, have an equal error of the opposite kind (over-correction), and when the objective and eyepiece are combined, a perfect correction is obtained.

The apochromatic objectives are 'under-corrected,' while the achromatic objectives of low power are 'over-corrected.' The compensating eyepieces for the former are over-corrected, and the Huyghenian eyepieces for the latter under-corrected. With low powers of the achromatic type, the compensating eyepieces are disadvantageous; but with high powers, where the defects caused by the hemispherical front lens give rise to error identical with that in the apochromatic objectives, and which the compensating eyepieces are designed to overcome, these special eyepieces can be employed, but the result is not sufficiently beneficial to justify the purchase of them for use with high-power achromatic objectives only.

A most advantageous feature is imparted to the Zeiss compensating eyepieces. They are all designed to work in the same focal plane, so that when two eyepieces of this series of different powers are interchanged in the body of the microscope no alteration in the focusing is necessary.

A number is engraved on each eyepiece, which, multiplied by the initial magnifying power of the objective, will, when used at the tube-length for which the eyepiece is designed, indicate the magnifying power that is being employed.

The compensating eyepieces designed for the 6-inch

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tube-length can be used on the 10-inch tube, and those for the 10-inch at the 6-inch tube-length, without detrimental effect; but in the former case about half must be added to the product of the multiplication of the power of the objective and eyepiece, and in the latter case about one-third must be deducted, in order to arrive at the magnifying power (see *ante*, p. 69).

Holoscopic and Universal Eyepieces.

These eyepieces are made by J. Swift and Son and Watson and Sons, and are intended to be used with objectives of both the apochromatic and achromatic types. The lenses used are made of a selected optical glass, which produces a degree of over-correction similar to that associated with compensating eyepieces when the separation between the eye and field lenses is increased. In order that this may be conveniently effected the eye lens is attached to an inner, or draw-tube, sliding inside the outer tube which fits the microscope body. When the eyepiece is closed together it becomes of the Huyghenian type; when the eye lens tube is pulled out, it gives the effect of a compensating eyepiece. The amount of over-correction can be exactly obtained by the greater or lesser extension of its draw-tube, a scale being provided to record results. This eyepiece is, therefore, applicable to all classes of objectives, and, being made in a useful range of magnifications, will be found a desirable pattern to start with, and acquaintance with its working will lead to greater appreciation of it. It yields excellent effects photographically. Even if it be intended to limit the equipment to achromatic objectives, this type of eyepiece will generally be found to present points of superiority over the ordinary Huyghenian pattern.

FIG. 21.—HOLOSCOPIC EYEPIECE.

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Kellner Eyepieces.

This is an achromatic form of eyepiece, giving an exceedingly large field, which is considerably used for the examination of animalculæ, pond life, etc. A certain amount of definition is, however, sacrificed in working with it, and although occasionally of use, we should recommend the microscopist, before purchasing any, to judge for himself as to the desirability or otherwise of his having them; they are not by any means necessary adjuncts.

Projection Eyepieces.

These were designed specially for projecting objects on a screen and for photographic purposes. They give an exceedingly small field, but an exquisitely sharp one. In order to obtain good results with these, it is necessary to alter the position of the eye-lens until the image of the diaphragm appears sharply projected upon the screen. For this purpose the eye-lens is mounted in a tube having in it a spiral slot, permitting of the eye-lens being moved to and fro with great precision. They are usually made in four powers, magnifying 2 and 4 diameters respectively for the short tube-length, and 3 and 6 diameters for the English tube-length. The most serviceable are the 4 for the short tube and the 3 for the long tube. All photo-micrographers of note use these eyepieces, and they can usually be employed to advantage, even with low-power achromatic objectives of good quality. For photographing with ordinary objectives of low power, the 'A' eyepiece gives good results.

FIG. 22.—PROJECTION EYEPIECE.

Binocular Eyepieces.

As mentioned under 'Binocular Microscopes,' neither of the leading Continental firms make the binocular micro-

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scope." For those, therefore, who desire to be able to employ both eyes, they make a binocular eyepiece, but only for the Continental length of tube, and this should be particularly understood. The one with which we are acquainted is that designed by Abbe and manufactured by Zeiss, as shown in Fig. 23. In some hands it gives very beautiful results, while other workers have failed to derive advantage from it. It is designed to give stereoscopic effects and to work with both high and low powers. If it were mounted in some lighter manner it would perhaps become more generally used; its weight is very much against it when working with high powers; still, for the advantages it affords it is an adjunct which is by no means to be despised.

Blank Eyepiece.—It will be found convenient, especially in examining the back of the objective to observe diffraction phenomena, cones of illumination, etc., that a blank or 'dummy' eyepiece be employed; that is, an ordinary eyepiece mount having no lenses in it. The aperture in the cap must, however, be a very small one.

STANDARD GAUGES FOR EYEPIECES.

The following sizes were adopted by the Council of the Royal Microscopical Society on December 20, 1899, as the standard inside diameters of draw-tubes for microscopes, the tightness of the fit of the eyepiece being left to the discretion of the manufacturers:

No. 1, ·9173 inch = 23·300 millimetres.
No. 2, 1·04 inches = 26·416 ,,
No. 3, 1·27 inches = 32·258 ,,
No. 4, 1·41 inches = 35·814 ,,

No. 1 is what is known as the Continental size, which is made almost universally by Continental manufacturers, and has been supplied for many years. It has also been largely used by English manufacturers.

No. 2 is the mean of the sizes used by the English trade for students, and small microscopes.

No. 3 is the mean of the sizes used for medium-sized binoculars and for microscopes of a similar class.

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No. 4. is the maximum size for long tube binoculars.

It will be admitted that the makers here have an excellent choice of gauges for their instruments, and it is eminently desirable that eyepiece fittings be confined absolutely to these sizes. For many years it has been the rule with certain houses for reasons of their own to make draw-tube

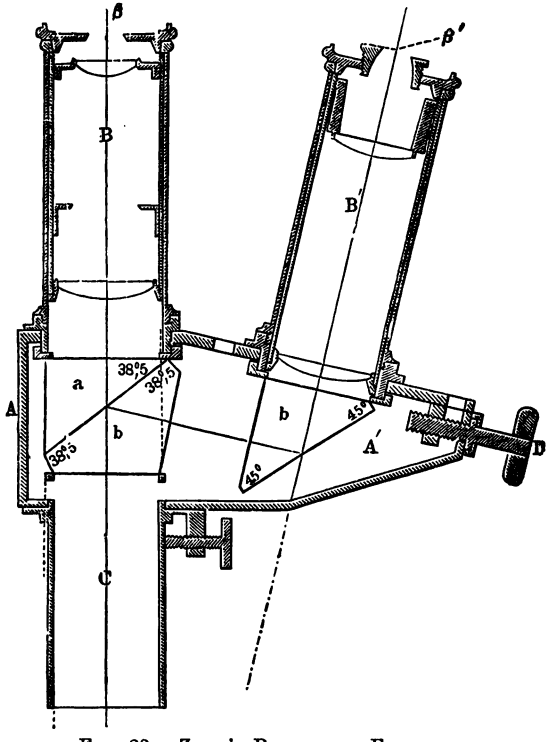

FIG. 23.—ZEISS'S BINOCULAR EYEPIECE.

fittings of diameters which do not conform to either of the above-mentioned standards, and those who may be contemplating the purchase of a microscope will be conferring a benefit on microscopy generally and directly on themselves if they insist that the microscope they select shall have one or other of the standard sizes of fittings for eyepieces, and refuse to accept any other diameter.

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CHAPTER III

ILLUMINATION AND ILLUMINATING APPARATUS.

Monochromatic Light and Light Filters.

Absolutely monochromatic light is a light of one refrangibility—that is, a colour of one uniform wave-length. As used in microscopy, monochromatic light means light with a small range of refrangibility, and it is important that its function should be clearly understood.

If white light is divided into its component parts by means of a prism or a spectroscope, a regular band of colours is produced, termed the spectrum, commencing with red at one end, followed by orange, yellow, green, blue, indigo, and finishing with violet. In physical optics light is regarded as travelling in waves, the amplitude of each of which is very small, compared with the wave-length—not more than about 1 : 10,000. Now, the length of a light wave varies according to the portion of the spectrum that is used. At the extreme red end of the spectrum it measures ·76 μ,* and the wave-length decreases through the range of colours until at the extreme violet end it measures ·39 μ. From this it will be seen that nearly double the number of waves of light would be oscillating per millimetre with a violet light than with a red.

The numerical aperture of an objective is increased by

* $\mu = \frac{1}{1000}$ of a millimetre, and is called a micron. There are 7½ to 8 microns in the diameter of a human blood-disc.

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the use of a dense medium enveloping the object and the front lens of the objective, as we have seen by the description of immersion objectives. The oil or other medium employed shortens the wave-length of the light used, whatever its colour, and when we use a light of shorter wave-length than it would have when passing through air, we increase the effective aperture of the objective. Accordingly the resolving power of a lens is increased by shortening the wave-length of the light admitted to it, and this is accomplished in either of two ways—(1) By employing blue instead of white light, or (2) by converting the lens into an immersion lens, and interposing a layer of oil instead of air between it and the object. For instance, if a microscopic objective were used with white light, and its limit of power to resolve fine structure were 50,133 lines per inch with such illumination, its limit would be 54,342 lines per inch with monochromatic blue light (line F).

A natural conclusion from these statements would be that the farther towards the violet the monochromatic light were used, the more marked would be the results obtained; but although this is correct theoretically, it is not true practically. Microscopic objectives are corrected for visual purposes for use with the brightest rays of white light, and if the extreme ends of the spectrum were employed—the objective not being calculated for these—rise would be given to spherical aberration, even in the best objectives, preventing the accomplishment of good work. If a lens were corrected for spherical aberration when used with light from the extreme blue end of the spectrum, under existing conditions of manufacture it would work at its best with the light for which it was designed, and if light lower down in the spectrum were employed, spherical aberration would be apparent. It must be borne in mind that light of extremely short wave-length is sensibly absorbed by glass, also the eyesight is not keen in extreme blue and violet lights, consequently the range of light that

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is practically available for monochromatic illumination is restricted.

Another advantage gained by the use of monochromatic light is, that as there is but one colour of the spectrum used, objectives of high-class make of the achromatic form are rendered practically equal to apochromatics by the removal of the secondary spectrum—that is, any chromatic aberration that may be present in the objective is annulled by the monochromatic light with which the illumination is effected. The more nearly the monochromatic light which is used approaches to that ray for which the spherical aberration in the objective is best corrected, the better will be the resulting definition. In some cases, so advantageous has this means of illumination proved, that, using two objectives of the same power alternately, one an expensive apochromatic, and the other an achromatic, it has been difficult to tell which was being employed.

True monochromatic light can be at the present time obtained by means of prisms only, and a very excellent apparatus is that designed by Mr. E. M. Nelson, and manufactured by Mr. C. Baker, of 244, High Holborn, London. It consists of prisms, slits, and condensing-lenses so arranged as to afford every facility for obtaining accurate effects.

Beautiful results with monochromatic light are to be secured by the use of a heliostat to reflect sunlight upon a slit and prisms.

Dr. G. Johnstone Stoney has devised a most excellent heliostat actuated by means of a lever clock movement, and with fine adjustments by means of Hook's handles for setting, and a plane-parallel worked mirror. The brilliance of illumination secured by such means is very intense, and a spectrum 2 feet in length can be easily secured if the microscope be placed at some distance from the prisms. If the spectrum be allowed to fall on a sheet of white paper or cardboard at the position in which the microscope is placed, the exact tint of illumination that is required can be selected,

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and that alone utilized in the microscope. The use of a heliostat is necessarily limited in Great Britain, and especially so in London, on account of the few hours of bright sunlight that are available, but in countries where this restriction does not apply the heliostat is particularly to be recommended, not only for this especial purpose, but also for general microscopical work and in photography.

Many experiments have been made with a view to producing monochromatic light screens by means of pigments and the combinations of coloured glasses, and thereby obviating the necessity for prisms. So far these attempts have been only partially successful, all that have been made passing light of more than one colour, but it is to be hoped that the desired result may yet be achieved. Very material assistance is, however, afforded by these screens, not alone for actual monochromatic illumination, but also for general work, for they allow of a large cone of illumination being utilized without discomfort to the eyes. They are usually placed beneath the sub-stage condenser, and are employed in photo-micrography very considerably for neutralizing non-photographic colours in objects, and rendering the actinic and visual rays in an objective more nearly coincident, also in visual work for minimizing light glare with large cones of illumination. This latter applies equally to apochromatic and achromatic objectives, the screen often producing stronger contrast and a crisper image than could be obtained without one.

The most advantageous colour for all-round visual purposes is the green-blue, and excellent light screens are made on a plan, originally suggested by Mr. J. W. Gifford, consisting of a film of gelatine stained with malachite green deposited on a circular disc of signal green glass, and having a protecting cover-glass for the gelatine fixed by means of a ring of cement. Fluid screens can also be made with great accuracy, and with care will last a considerable period, but it is particularly essential that they be protected from light, or they are liable to fade. The Gifford's screen referred to

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above, in a fluid form is made by mixing a small proportion of malachite green in glycerine in a trough. The light from the illuminant that is to be used is examined spectroscopically through the medium in the trough, and the coloured fluid is added until the red end of the spectrum is absorbed; if this be done exactly a minimum of loss of light occurs.

Another excellent screen is produced by making a saturated solution of acetate of copper, but a trough with an opening of at least $\frac{1}{8}$ inch back to front is necessary to obtain an effective colour with this fluid.

For photography, discs of light and dark yellow and various shades of blue glass are constantly employed. The great desideratum in a light screen is that it shall pass a large quantity of light. No doubt, mixtures could soon be made that would produce monochromatism, if only great transparency were not of importance.

Mr. Nelson states* that monochromatic blue light 'makes a difference of about 14 per cent. in the case of low apertures, but beyond those of 0·9 N.A. its influence in increasing resolution is so small as to be hardly worth taking into account. What it does effect is the sharpening and clearing of detail already resolved.'

This is no doubt because only a small part of the first diffraction image, seen when looking at the back of the objective with the eyepiece removed, is utilized by the lens, and that this part consists of blue light whether it be blue or white light that is employed for illuminating, so that in both cases there are the same materials for resolving the detail of the object, and the only difference is that there is a haze of light of lower refrangibility (or greater wave-length) also present, which forms a luminous veil over the whole field. It is this veil which is removed by using monochromatic light, and therefore the effect is to sharpen and clear the detail that is already resolved.

* *Journal of the Royal Microscopical Society*, 1893, p. 15.

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Sub-stage Condensers.

A few years since all that was considered necessary in order to illuminate in a proper manner an object under examination was the mirror, perhaps in conjunction with the bull's-eye stand condenser; and in many cases the mirror was hung on a tailpiece which could be moved in an arc round the centre of the stage, and by this means light at any angle could be reflected on the object. The day for this, however, has gone by, and anyone who requires to get even fair results must use a sub-stage condenser in some form or other. Especially does this apply to high-power objectives. Plenty of illumination can be obtained with the mirror only for low power objectives, but beyond these the object becomes ill-defined and the field dark. More especially since the study of bacteriology has taken so prominent a position has the condenser come to the front. Without its aid it would be almost impossible to distinguish between different species of these minute organisms. To the leading members of the Royal Microscopical Society, and especially to Dr. Dallinger and Mr. E. M. Nelson, is due the steady improvement that has taken place in the optical qualities of the sub-stage condenser. The two gentlemen named have been indefatigable in their appeals and demonstrations to microscopists, urging the pre-eminent position that it should occupy in manipulation, and the proper methods of using it.

One of the most largely used of condensers is a chromatic one, named the Abbe illuminator, originated by the firm of Carl Zeiss, and now supplied by nearly all opticians. It is made in two forms, one having a numerical aperture of 1·20, and the other of 1·40. The former is the more commonly employed, and is principally adopted by students. The

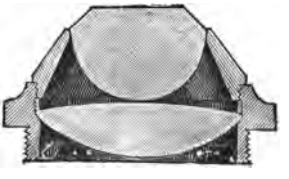

FIG. 24.—ABBE ILLUMINATOR. (1·20 OPTICAL PART.)

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optical portion is shown in Fig. 24. It gives a brilliant illumination, with the highest power objective, while with the lower powers, by removing the top lens, good results can be obtained. Its price is low, but it has the great disadvantage of not being achromatic, and having so large an amount of spherical aberration as to be almost useless for critical work, for although its total aperture is large, its aplanatic aperture is less than ·50. Nevertheless, it fills a distinct position in microscopical work, and is very easy to use.

The Abbe illuminator was followed some years later by an achromatic condenser, having a numerical aperture of 1·0, which also originated with the firm of Carl Zeiss, and was subsequently made by several London opticians. This was a substantial improvement on the older chromatic pattern. It, however, had the disadvantage of being somewhat large and heavy, and yielded an aplanatic aperture of less than ·70. The necessity for using a condenser that yields a solid cone of illumination at least $\frac{3}{4}$ of the total aperture of the objective with which it is to be worked has become universally recognised, and has resulted in the introduction by all the leading London houses of condensers which possess no disadvantage as compared with the Abbe condensers, while their efficiency is enormously greater.

One reason why the Abbe condensers have enjoyed popularity has been the facility with which they can be used, and this was in a considerable measure due to the field lens being of a large size. Several condensers with large aplanatic apertures, but with comparatively small field lens, have been introduced, but Mr. C. Baker, of 244, High Holborn, was the first to offer to the microscopist a sub-stage condenser, which he described as a modification of the Abbe achromatic, with a field lens of sufficiently large diameter ($\frac{7}{8}$-inch) for quick and easy use. In this condenser the aplanatic aperture is ·90 and the magnifying power $\frac{4}{10}$-inch. It is shown in Fig 25.

This was quickly followed by a condenser of similar

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external design by W. Watson and Sons, which they named the Universal Condenser, but constructed on the principle of their holoscopic objectives with a triple back lens of ·92 inch diameter. The aplanatic aperture of this exceeds ·9, the power is $\frac{4}{10}$ inch. These two condensers are unquestionably the best obtainable at the present day for the amateur and he who does not do exclusively high power work, and, although the cost is somewhat greater than that of the chromatic Abbe illuminator, the additional expenditure will be more than repaid by the fine work that will be possible.

There is one other point with regard to these condensers,

FIG. 25.—C. BAKER'S ACHROMATIC CONDENSER.

and that is that the mounting, which in the Abbe condensers was always large and somewhat restricted the movements of the plates of a mechanical stage, is reduced to considerably narrower dimensions, and the objection referred to is almost entirely removed. Amongst high-power condensers special mention may be made of Beck's oil immersion, Swift's pan-aplanatic, and Watson's para-chromatic (dry) and holoscopic (oil immersion). No achromatic condensers of the types named can be obtained from any Continental manufacturer.

Messrs. Powell and Lealand are entitled to special commendation for their early appreciation of the value of condensers having large aplanatic apertures, and they were many years in advance of other makers in the production

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of such condensers. They designed two of apochromatic form, one having a N.A. of 1·0 for use dry and the other an oil immersion having a N.A. of 1·40. Some of the condensers before referred to possess aplanatic apertures slightly in excess of those by Powell and Lealand, but they are beautifully corrected, and those who may be wishing to have condensers in keeping with their apochromatic objectives would find these admirable. Still, the value of a sub-stage condenser is not to be reckoned by its total numerical aperture, but by the solid cone that it will transmit, or, in other words, by its perfection of correction ;

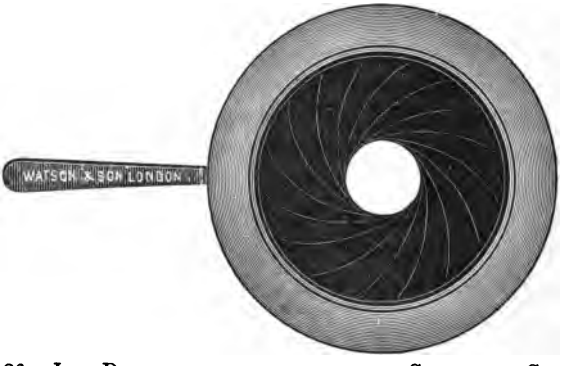

FIG. 26.—IRIS DIAPHRAGM AS FITTED TO THE CONDENSER CARRIER.

for its aplanatic cone alone can be employed for critical illumination.

If, therefore, an achromatic condenser gives a superior aplanatic cone to an apochromatic for practical work, it may generally be considered preferable, and, seeing that this is done, and that the achromatic form is very much less costly than the apochromatic, the microscopist may with assurance take the former, and be satisfied that he can perform with it all that possibly can be done.

In Fig. 25 the achromatic condenser that is shown is mounted on a carrier for the sub-stage. It is provided with an iris diaphragm similar to that illustrated in Fig. 26, by means of which any desired aperture may be quickly and

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exactly obtained. It will be found of utility to have the arc, through which the lever controlling the iris diaphragm travels, provided with a scale of divisions, so that results may be quickly reproduced or any special aperture may be obtained; but for this purpose it is necessary that the diaphragm shall respond immediately on the pressure of the lever handle; there must be no loss of time in the movement. The carrier is further provided with an arm, having a rotating cell, in which may be placed stops for producing dark ground illumination in the same manner as with the spot lens, described on page 101; also stops for obtaining oblique illumination for the resolution of the markings on diatomaceæ, and for holding tinted glasses or light screens. This form of carrier is applicable also to the Abbe illuminator.

A very efficient condenser can be oftentimes formed by fitting a low-power objective into a suitable carrier in the sub-stage. A small iris diaphragm, called the Davis's Shutter, having the universal male Society's screw at one end and a female screw at the other, together with a tube, fitting into the sub-stage and provided with the universal thread, into which the iris diaphragm may be fitted, is a very suitable carrier for the objective.

THE APLANATIC APERTURE of a sub-stage condenser is ascertained in the following manner:

The condenser is accurately centred, and both it and an objective are focused in the usual way on an object mounted in *Canada balsam*, the edge of the lamp-flame being employed. We will presume that the objective has a numerical aperture of ·5 N.A. The full aperture of the condenser is then used, and the object so placed that the balsam portion is still between the condenser and objective, but the object itself is not in the field. It would be well that a balsam-mounted object were always used for this experiment, as the result is slightly affected by different media. The eyepiece is now removed, and the back lens of the objective is examined. It may be found that it is completely filled

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with light, as in Fig. 27, under which circumstance the condenser has an aplanatic cone exceeding the N.A. of the objective. The aperture of the condenser can now be limited by means of a diaphragm, and an approximate value obtained for the size of diaphragm that is used. The edge of this diaphragm should be so set that its edge is just seen appearing at the margin of the objective as in Fig. 28. The aperture of the condenser when used with this size of diaphragm is therefore a shade less than N.A. ·5—say ·45. An objective having a larger N.A., say ·95, is now employed, and it will be found that the back lens of the objective is no longer filled with light. Theoretically, this is the condition under which the aplanatic aperture should be estimated, but when a flat flame of a lamp is presented edgewise, its

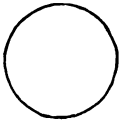

FIG. 27.

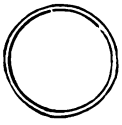

FIG. 28.

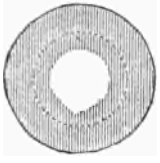

FIG. 29.

FIG. 30.

image has corresponding depth, and when one part is focused on the object, other parts of the image of the flame will necessarily be out of focus. There is therefore a certain range of adjustment of the condenser within which the effect (so far as it depends on focusing the light on the object) will be pretty much the same. But these different positions give different apertures to the condenser as judged by the light reaching the back lens of the objective. The condenser should then be gently racked upward until the disc of light is at its largest (Fig. 29); until on a further movement of the condenser two black spots appear, one on either side of the middle of the disc (Fig. 30), which increase as the condenser is further racked up. *The last point before the appearance of the black spots furnishes the position in which the condenser has the largest aperture*

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consistent with its outstanding spherical aberration not too much interfering with the highest results, and is the limit of the condenser for critical work. Any further advance of the condenser gives merely annular illumination, which, of course, is to be avoided, excepting when stops are used.*

How to use the Condenser.

The condenser requires as much care and skill in adjusting as the objective, for if it be improperly set up it will give rise to 'false images.' For an objective to work at its best, it will be necessary to focus the image of the *edge* of the lamp-flame sharply upon the object on the stage, and this, with the modern condensers of large aperture, requires to be as accurately performed as the focusing of the objective upon the object; hence the value of the fine adjustment to the sub-stage (see page 17). The following will be the procedure:

A proper microscope lamp, as described on page 107, should be set in front of the instrument with the edge of the wick towards the microscope, and the light from the lamp may be allowed to fall directly upon the condenser, or a plane mirror may be used. The sub-stage condenser should now be centred, first having been placed in about the position that it will occupy when focused. The centring cannot, of course, be properly effected without a centring sub-stage; but where there is only a fixed under-fitting, it is well to set the condenser at the position where it is most central. It is understood that the under-fitting is centred with the optical axis of the microscope when sent out by the makers; but owing to the fitting-tubes being more or less elliptical, it often happens, if the condenser is rotated in the under-fitting, that it will be central in one position only, and at this position it should be placed for working when there are no centring screws. Some condensers have

* E. M. Nelson, *English Mechanic*, November 16, 1888 (vol. xlviii. No. 1,234), and 'The Microscope and its Revelations,' by Dr. Dallinger.

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fitted on top of them a removable cap with a very small pin-hole. This pin-hole should be focused with, say, an inch or a ½-inch objective, and the condenser centred by it; the cap should then be removed from the condenser for working. Condensers not provided with this cap can, as a rule, be centred by using a diaphragm having a very small aperture at the back of the lenses, and focusing the aerial image of it with a ½-inch objective; but the easiest way of centring is to make a very small spot in the middle of the top of the lens with a pen and ink; centre by this spot, and wipe it off. It will not make any difference to the performance of the condenser, and will ensure accuracy and save time. Having centred the condenser, it should be racked up until it touches the under-side of the slide, the objective being made to touch the cover-glass of the object on the upper side; see that the diaphragm of the condenser is open, reflect the light with the mirror, and thus illuminate the field; then rack the microscope body upwards until the object comes into view. If it is found that there is too bright a flood of light, the aperture of the condenser must be decreased a little by using a smaller diaphragm. Having focused the object on the upper side with the objective, it will be necessary to focus the condenser. Rack this downwards from the object very slightly until the image of the lamp-flame is seen in the centre of the field, the remainder being comparatively dark, as in Fig. 31. If now it be desired to have the whole field equally brilliant a bull's-eye stand condenser may be interposed between the lamp and the mirror, the plane side of the bull's-eye being towards the lamp; or the burner of the lamp may be turned round till the flat of the wick is towards the mirror. Where high powers are to be used, the object to be examined may with advantage be set upon the stage and

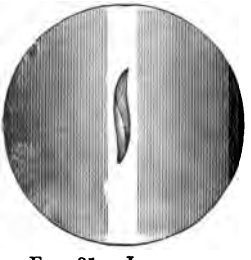

FIG. 31.—IMAGE OF LAMP-FLAME.

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focused with the ½-inch or other low-power objective, and the sub-stage condenser focused upon the object. The high power may then replace the low power, and the condenser will be in adjustment. If it be found that the image of the lamp-flame is not in the middle portion of the field on exchanging the objectives, it will show that the objectives have not exactly the same centres, and the image must be set central with the high power by altering the position of the condenser by means of the centring screws of the sub-stage.

The next question is, What amount of light should be admitted from the condenser in order to see the object at its best? Mr. Nelson has suggested that the aperture of the condenser should be about three-quarters that of the objective, and in order to arrange this it will be necessary to remove the eyepiece from the microscope, and look down the tube at the back of the object glass, opening the diaphragm of the condenser to its fullest extent. Bearing in mind the size of the circle of light seen, gradually diminish the opening of the diaphragm of the condenser until one quarter of the back lens of the objective is shut out; again put in the eyepiece, and the desired amount of illumination is arranged. The aperture employed should be varied slightly according to the transparency or opacity of the object under view.

When the condenser is centred and focused, and the back lens of the objective is three-quarters filled with light, a *critical image* is obtained; that is, the objective is understood then to produce the finest results it is capable of.

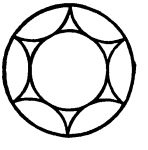

FIG. 32.—BACK LENS OF OBJECTIVE.

Mr. Nelson's ¾-cone method of illumination has been almost universally accepted as a most practical one; but the following plan, which was suggested to the writer by a microscopical friend, has given very satisfactory results. On examining the back lens of the objective with a striated object, such as *Pleurosigma angulatum*, resolved and focused

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upon the stage, there will be seen a central disc surrounded by six diffraction spectra similar to Fig. 32. With the $\frac{3}{4}$-cone of illumination, the surrounding spectra will, in some cases, appear to overlap the central disc, and in others will not appear to touch it. Our plan is to open the diaphragm of the condenser only to such an extent that the spectra just touch, but do not overlap, the central disc. This would necessitate that in some instances we should employ rather less than a $\frac{3}{4}$ cone of illumination and in others rather more than a $\frac{3}{4}$ cone. We have not been able to observe that any loss of resolution results from this practice; but, on the other hand, in our opinion detail is more clearly seen, and appears crisper under these circumstances of illumination than any other. This system is especially advantageous when monochromatic light is used.

It will be found of great advantage to become acquainted with the appearance of the back lens of the objective when working; many hints of importance may be gleaned from it, enabling manipulation to be effected with increased precision. For this purpose the 'dummy' eyepiece referred to on page 82 is a most useful adjunct.

When working with monochromatic light, the condenser must be focused so that the whole of the light which is visible on the back lens of the objective when the eyepiece is removed shall appear as nearly as possible of the same colour.

Condensers having a numerical aperture of 1·0 N.A. and over require to be immersed in order that they may work at their full aperture; that is, a drop of immersion oil or Canada balsam must be placed between the top lens of the condenser and the object. It will be found generally that the condenser is a little too long in focus for continuity between the top lens of the condenser and the underside of the object to be maintained. Under such circumstances an additional thin 3×1 inch slip, or a piece of cover-glass, should be placed under the object, which will enable the

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oil contact to be maintained. The distance between the condenser and the object will vary according to the thickness of the slip on which the object is mounted, and the intermediate contact glass will have to be selected accordingly. To use an oil immersion condenser effectively the object must be mounted in some medium, and not dry upon the cover-glass.

As before mentioned, with the majority of condensers stops are supplied having the centres blocked out, as shown in Fig. 33, by means of which dark-ground and oblique illuminations are obtained. Dark-ground illumination gives a most beautiful effect to very transparent objects, such as infusoria, pond-life specimens, etc. In the form of carrier for condensers shown on page 91, a cell is provided just above the iris diaphragm to carry the stops. One similar to *a* (Fig. 33) is placed in the cell, the iris diaphragm is opened completely, the condenser having been previously adjusted in the usual way, when it will be found that the object will be illuminated, but the ground on which it is seen will be black. Different objectives require stops of special sizes, which may be readily made of blackened cardboard, cut to the most suitable size for working with the objective.

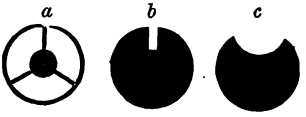

FIG. 33.—STOPS FOR CONDENSERS.

These stops can be further used for strengthening the contrast in the image with large cones of illumination and objectives having high apertures. This method does not minimize in any way the effective working of the objective, for with objectives of large aperture rays may be present which only impart brightness to the field, but do not contribute to making visible the fine detail upon the object. If less than half of the lateral spectra, as shown in Fig. 32, are seen on looking down the tube at the back lens of the object glass with a striated object in focus, then the central portion of the direct beam or central disc has no lateral image corresponding to it in the portions of the spectra

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that are visible. Under these circumstances that central portion of the central disc in no degree contributes in enabling the detail to be seen, but only produces a haze; by blocking it out the haze is removed, and there is a great improvement in the resulting definition. This produces oblique illumination in all azimuths.

THE CHOICE OF A CONDENSER.—That the condenser is an absolute necessity cannot be too strongly impressed. No good results can be obtained without it.

Condensers, like objectives, not only vary in aperture, but also in power, and the higher the power of the condenser the smaller will be the image of the lamp-flame that it transmits. Consequently, if a condenser of high power is used with a low-power objective, the illuminated portion of the field will be exceedingly small, while if a low-power condenser is used with a high-power objective, the image of the lamp-flame is so magnified that the whole field is bright, and it is not easy to tell when the condenser is exactly focused. Furthermore, under such circumstances as the latter, it is impossible to get the best effect with the objective. It has usually been recommended that two condensers, one of high and the other of low power, should be included in a complete equipment, but the new types of condensers previously referred to cover such a large amount of ground, that the average microscopist really requires only one. Choose a condenser that gives an aplanatic cone of ·90 N.A, and if the major portion of the work is to be with objectives of low and medium magnifications, one of low power should be selected; if principally with high powers, a corresponding high-power condenser will be necessary. It must be remembered that by removing the uppermost lens by unscrewing its cell, the remaining combinations of a high-power condenser form a very serviceable low-power condenser.

Oil immersion condensers are of especial utility where objectives of the largest aperture are employed, and these, again, in several instances work well as dry condensers, and

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with the top lens removed become low-power condensers of moderate aperture.

If the advice given be followed and the condenser used intelligently, reliance will be placed in the work performed and far superior results secured than would be possible with a badly corrected chromatic condenser.

The Spot Lens.

Before the sub-stage condenser came into general use the spot lens and paraboloid were largely utilized for obtaining dark-ground illumination. They have, however, been to a considerable extent superseded, owing to the perfection in which the same effect can now be obtained with the condenser. They, are, nevertheless, often employed, and preferred by some to the condenser. It must be understood that they cannot take the place of the condenser for ordinary direct illumination. The method of using is simple, the spot lens being intended for low powers up to ½ inch, and the paraboloid for higher ones. With both of them a plane mirror and the flat of the wick of the lamp should be used. If the spot lens be employed, the sub-stage that carries it should be moved up and down until a perfectly black ground is obtained; if additional brilliancy is required on the object, a stand condenser interposed between the lamp and the mirror, with the convex side of the condenser towards the mirror, will give a brighter effect. The paraboloid is adjusted in a similar manner, but instead of having a fixed black spot on the top of the lens it has an adjustable one, and the pin carrying this black spot should be moved up and down until the best effect is obtained. This latter is far more expensive than the spot lens, and the advantage gained is so slight that we can hardly recommend it.

For one class of work the spot lens is especially advantageous. Most sub-stage condensers have a very short focus, and if organisms in water in a trough are being examined, it is impossible to focus the condenser accurately

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through the trough and its contents. A spot lens has a longer focus, and gives under these circumstances the best results.

The Polariscope.

This consists of two parts, each composed of a Nicol prism of Iceland spar in a suitable mounting—one called the polarizer, which fits into the sub-stage, and the other the analyzer, which is usually inserted between the nosepiece of the microscope and the objective. By its means light is split up into its component parts, and most beautiful colour effects are obtained. The polarizer has a flange beneath, by which it can be rotated, and in this way the

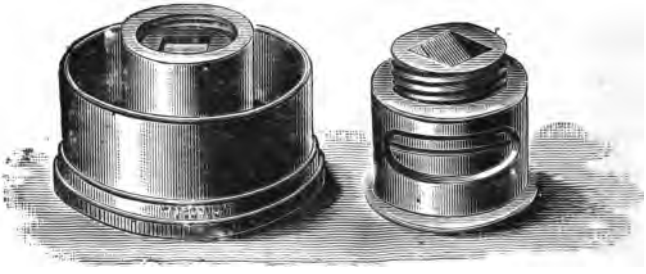

FIG. 34.—POLARIZER. FIG. 35.—ANALYZER.

colours are varied. In examining certain chemical crystals, geological slides, etc., it brings into view structure which without it would hardly be detected, and for this it is largely used in analytical work. In some instruments the analyzer prism is fitted in the body. This is rather an inconvenience unless the instrument be designed especially for petrology. For a binocular microscope, however, if it is placed between the nosepiece and the objective, it causes a separation between these two, which interferes with the performance of the binocular prism; because the closer the back lens of the objective can be brought to the binocular prism, the more perfect will the vision be. Under these circumstances the monocular tube only is generally used; or the analyzer prism can be mounted over the top

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of the eyepiece of the monocular tube. For use with the polariscope, varieties of tints and a back-ground of colour can be obtained by the employment of selenite films. These, in the cheapest form, are mounted in the same way as ordinary microscopic objects; but a still greater variety of effect can be obtained by having selenites fitting into a carrier to come between the polarizer and the stage in a sub-stage microscope. We illustrate one (Fig. 36) by R. and J. Beck. In this form each of the selenites is provided with a ring which rotates. The three being one over the other, either two or all three can be rotated together or in opposite directions to one another, and the effect is most striking. An inexpensive modification of this is made by Swift and Sons and others, called the mica-selenite stage, as shown in Fig. 37. This consists of a film of mica made to rotate

FIG. 36.—DARKER'S SELENITES.

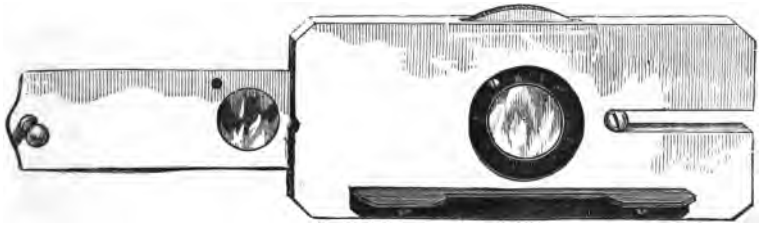

FIG. 37.—MICA-SELENITE STAGE.

in a brass plate, upon which the object is laid, and beneath it is a carrier with three separate selenites. These can each be pushed separately beneath the mica and the latter rotated. By this means all the different tints obtainable with any number of selenite films can be produced. It can be employed on any microscope. To get greater brilliancy the polarizer can be made to fit into the sub-stage con-

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denser on the under side, and the Abbe chromatic and achromatic condensers referred to previously are particularly suitable for this arrangement.

The Bull's-eye Condenser.

Many objects, being opaque, cannot be viewed by light from beneath, and consequently have to be illuminated from above. In order to do this a bull's-eye condenser is necessary. This usually consists of a plano-convex lens mounted on a stand, as shown in the figure. This has a ball and socket joint and a sliding telescopic tube, by means of which the lens can be placed in any desired position. The plane side of the lens should be turned towards the object, and the convex towards the source of the illumination, whether it be daylight or artificial by lamp. Latterly Mr. Nelson has suggested improvements in the construction of bull's-eye condensers in order to reduce the large amount of spherical aberration which is a necessary accompaniment of the single lens. His improved form consists of either two or three lenses in combination, and the advantage obtained, especially in photography, is well worth the additional outlay. The bull's-eye condenser is often of use in conjunction with the sub-stage condenser, to enable the field to be evenly illuminated with the latter, as mentioned on page. 96. It will be

FIG. 38.—STAND CONDENSER.

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found that the bull's-eye has to be placed rather farther from the lamp-flame ($\frac{1}{4}$ inch) when an ordinary glass chimney is used than with a metal chimney and mounting slip of glass.

The Parabolic, or Side Silver Reflector.

To enhance the effects obtained with opaque objects with stand condensers the side silver reflector will be found very advantageous. The arm on which this is mounted is attached to either the stage or limb of the microscope, or fitted between the nose-piece and the objective. The reflector consists of a highly-polished silver parabolic speculum. This reflector is placed by the side of the object, and light is thrown from the lamp through the bull's-eye on to its centre, and then thrown by the reflector on to the object. Most brilliant opaque illumination may be obtained by this means.

The Vertical Illuminator.

The merits of this piece of apparatus were but little appreciated until the study of metal surfaces microscopically was taken in hand. Its use had been almost exclusively restricted to ascertaining whether the specimens were mounted in contact with the under surface of the cover-glass—information which is of importance when an oil immersion objective is being employed on an object mounted dry, for if the specimen is not adherent to the cover-glass it cannot be seen at all with this illuminator. It is also used for the resolution of the markings on diatoms that are mounted dry on the cover-glass; in this case it is, however, only of value with immersion objectives.

In metallurgical work it has rendered permissible the examination of surfaces of metals with high-power objectives. The surface to be examined is highly polished and then etched with acid, liquorice juice, or other medium, as will be found described on page 187, and no covering-glass at all is used. It is made in two patterns, one with a prism,

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and the other with a disc of cover-glass. Metallurgists usually have the two forms, finding one more serviceable than the other under certain conditions and with different specimens. They both have their distinct value. Generally speaking, for this class of work the prism pattern is the better for low-power objectives, and the disc form for the high powers. The latter is illustrated in Fig. 39, and may be either built in the body of the microscope or screwed to the nosepiece of the microscope above the objective; the one illustrated is arranged for the latter. The disc of cover-glass attached to a little clip having a milled head *a* is placed through a slot in the fitting *b*, and set at an angle of 45° to the optic axis. Light is received on this disc of glass through the small opening in the body of the fitting *b*, and it is totally reflected through the object-glass on the object, the objective acting as its own achromatic condenser. In order that the light may be focused on the object, the lamp-wick from which the light is being obtained must be the same distance from the reflector as the latter is from the diaphragm of the eyepiece, if a positive eyepiece is being used, or to the eye lens, if a Huyghenian or negative eyepiece is employed.

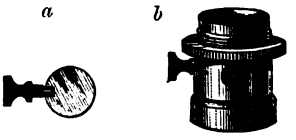

FIG. 39.—VERTICAL OR DISC ILLUMINATOR.

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CHAPTER IV.

ACCESSORY APPARATUS.

The Lamp.

It is most important that the microscopist should possess a good and suitable lamp, otherwise he cannot work to the greatest advantage. The amateur will often be found working with a reading-lamp or an ordinary oil-lamp, but good work can never be done conveniently by this means. There are two or three important points which must be borne in mind. In the first place, if light is proceeding from the one illuminating point only, and the remainder of the room is dark, while using the microscope, a great deal better effect can be produced than if the whole room be illuminated. In the next place, a small brilliant source of light is far better than a large one. In recent years special attention has been paid to this matter, with the result that lamps have been constructed with which the best work may be accomplished. The following are desirable features which should be embraced by a good microscope lamp: The reservoir for oil should be large in diameter and flat, so that the light may be brought down very close to the table. For this reservoir glass is usually preferable to metal, it being much cleaner, and the worker is able to tell when his oil is getting exhausted; whereas with a metal reservoir, unless careful reckoning is kept, in the middle of some important observation the light may go out from want of oil. A half-inch wick is generally found to be sufficient. We strongly deprecate the use of glass chimneys.

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They are always liable to get broken very easily, and become a source of expense, in addition to which, if away from town, there is a possibility of not being able to get the right kind, and so work may be delayed. Far better will be found the metal chimneys now made by nearly all opticians, with a carrier for a 3 × 1 or 3 × 1½-inch slip.

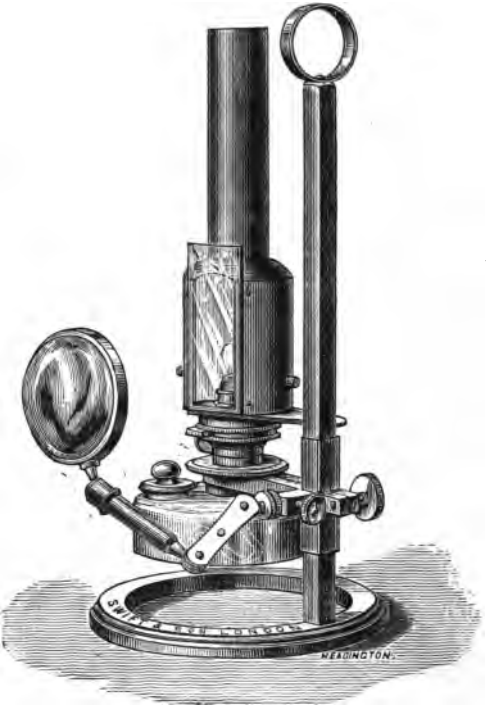

FIG. 40.—MICROSCOPE LAMP WITH METAL CHIMNEY.

It is obvious that if the slip be broken it can be immediately replaced, it being part of the microscopist's average stock. It is also desirable that the bar on which the lamp is raised and lowered on the stand should be a square one. If round in shape, the lamp is apt to swing round on the stand and the whole to topple over. This is an impossibility with the square bar. Such a lamp is shown in Fig. 40, by Swift

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and Son, and modifications of it can be obtained from most dealers.

A similar model of lamp which has rack-work and sliding adjustments in the vertical direction, and a quick acting screw for lateral adjustments, is also obtainable. Its mechanical arrangements enable the light from the lamp

FIG. 41.—ELECTRIC MICROSCOPIC LAMP.

to be shifted just the slight amount that is often needed, and obviate the necessity for moving the lamp itself, as would otherwise have to be done.

Electric Lamps.—The increasing use of electric light has caused some attention to be given to this form of illuminant for microscopical work. Figure 41 shows a very handy pattern that can be connected to the ordinary house

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circuit. The lamp should have a frosted bulb, and have a diaphragm, preferably of the iris form, placed beneath it. If the opening of this diaphragm be treated as the source of light, very good results can be secured.

Incandescent Gas Lamps.—A very brilliant and efficient illumination can be obtained from an incandescent burner on a table-stand of suitable height properly shaded. The reticulations of the mantle would render it objectionable, but this can be obviated by placing an iris diaphragm a short distance from the light in the same manner as is described for the electric lamp above, or a narrow slit would answer the purpose equally as well; the diaphragm or slit would then be focused by the sub-stage condenser, and treated as the source of light.

The Revolving Nosepiece.

Time-saving arrangements will often be found useful in work, and the nosepiece is one of these. This is a piece of apparatus which is screwed into the fixed nosepiece of the microscope, and is made to carry either two, three, or four objectives, termed respectively the double, triple, or quadruple nosepiece. Each of the objectives can in turn be rotated into the optical axis, thus saving the necessity of unscrewing an objective and screwing another on in order to get a variation of power. In hospitals, laboratories, etc., it is usual to have one of these fitted to nearly every instrument. A new pattern which is described as dust-proof has found special favour, rendering unnecessary the removal of objectives after use. One of these is illustrated (Fig. 42). They are to be had made of an aluminium alloy which is extremely light, reducing the strain on the body tube. Any microscope having the universal size of thread for objectives will carry a revolving

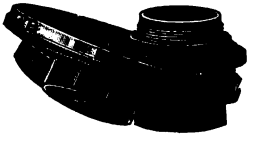

FIG. 42.—THE DUSTPROOF NOSEPIECE FOR THREE OBJECTIVES.

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nosepiece; no special adaptation is required. When the revolving nosepiece is screwed home, the objectives not in use must point towards the middle of the front of the stage, otherwise in rotating the objectives they are, with low powers, apt to foul the rack-work bar of the microscope.

The Nosepiece Iris Diaphragm, or Davis's Shutter.

This is a very compactly made iris diaphragm, which is placed between the nosepiece of the microscope and the objective. Its special function is to enable the aperture of an objective to be decreased, so that it may be used with dark-ground illumination, or to increase penetration when examining objects having several planes. For photographing opaque objects with low powers it enables the appearance of a small round object, such as a moth's egg, to be taken quite sharply. We have been shown photographs that were taken by a gentleman in America with a low-power objective, having an iris diaphragm fitted between the combinations of lenses, by the limitation of the aperture of which considerable depth of focus was obtained. The writer has not heard of this scheme having been tried in England.

FIG. 43.—DAVIS'S SHUTTER.

The Davis's shutter is furthermore very useful for examining and experimenting with the diffraction spectra seen on looking down the microscope tube at the back of the objective, when the eyepiece is removed and a striated object is being examined.

This iris diaphragm must have its aperture perfectly central and the threads quite true. The aperture of the iris, when completely opened, should be as great as it is possible for the inside of the mount of an objective to be, but the box of the iris diaphragm must not be so large as to

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touch the bearings in which the tube of the microscope is raised and lowered. It is well to have the lever of the iris diaphragm working in front of the body, and so that this may be easily arranged the male thread should be so mounted that it may be rotated, but should be very stiff in its rotary movement.

Camera Lucida.

This is designed to assist in drawing objects seen in the microscope. Photo-micrography has to a large extent superseded it; still, there are a great many who prefer this method to any other. Dr. Beale's neutral-tint reflector, which is supplied by all the opticians, is the cheapest and a very good form. It consists essentially of a neutral-tint glass—in which the image of the object is reflected—mounted in a frame to fit over the eyepiece. The method of using it is as follows: The microscope is set in a horizontal position, with the centre of the eyepiece 10 inches from the table. Illumination is arranged in the ordinary way. The cap of the eyepiece is removed, and the neutral-tint reflector is fitted in place of it, and is so arranged that the centres of the neutral-tint glass and the eye-lens of the eyepiece are in alignment, the former being set at an angle of 45°. On looking on this neutral-tint glass from the upper side, a disc of bright light will be seen on it, and if a piece of white paper be spread below on the table, on further examination the outlines of the object will appear to be upon the paper. If a pencil be now taken, the specimen can be sketched in its magnified form. This will be found somewhat difficult at first, nearly every worker seeming to find it necessary to work in some special manner of his own; but the secret of success is to arrange the balance of illumination by turning the lamp-wick up and down until a degree of light is found at which the pencil-point and image can be distinctly seen. When using students' microscopes, the eyepieces of which have no caps, it is usual to remove the eyepiece, fit the tube of the reflector to the outside of the top of the

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draw-tube, then reinsert the eyepiece and set the neutral-tint glass in position. The tinted glass is usually mounted on an arm which has a joint, so that it may be turned out of the way when not required without detaching the piece of apparatus from the microscope. The distance of 10 inches between the eyepiece and the table is maintained, whether the microscope has a 6 or a 10 inch tube-length.

The Beale's neutral-tint possesses the disadvantage of reversing the image that is seen with it. Mr. Ashe, therefore, devised a modification known by his name, which overcomes this defect, while maintaining the simple

FIG. 44.—ABBE CAMERA LUCIDA.

principle of the Beale's pattern. It is described in the Journal of the Quekett Club. Of more expensive description, but considered the best at present made, is the Abbe camera lucida (Fig. 44). The microscope may be used in a vertical or any inclined position with this apparatus. Its construction and the manner of using is as follows: Mounted in a cap, which is fitted immediately above the eyepiece, are two right-angle prisms; these are cemented together and form a cube. One of the cemented surfaces is silvered, but a small central disc is left clear, through

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which the object is viewed in the ordinary way. The prisms are so set that the image of the paper on which the drawing is to be made, and which is reflected by a mirror to the prisms, is by them conveyed to the eye. Thus the pencils of light reach the eye coincidently from both the microscope and the paper, and when drawing the object the pencil-point appears in the field of view very distinctly, and the minutest details can be exactly traced. Low-power eyepieces should be used with this camera lucida. There is another very commonly-used form of camera lucida, invented by Wollaston. In employing this it is necessary to put the microscope in a horizontal position, but it is somewhat difficult to see the pencil-point with it when working. To do this it is necessary that one part of the pupil of the eye shall be over the prism and receiving the image, while the other part is looking down on the paper below. A little practice soon enables one to do this. Some workers prefer this to Beale's neutral-tint reflector, but in our hands no superiority has been apparent in it. There are many other forms peculiar to individual makers, possessing more or less merit, some of which may be used with the tube in any position, the Swift-Ives pattern, made by Swift and Son, being particularly efficient; it can be used with the microscope set vertically or inclined.

The Measurement of Objects.

There are three ways in which this may be effected:

1. By having the stage divided—applicable to mechanical stages only.
2. By means of a camera lucida and a stage micrometer.
3. By means of eyepiece and stage micrometers.

1. If the movements of a mechanical stage are divided and read by verniers to very small parts of an inch or millimetre, the measurement of an object can be effected by having in the eyepiece a disc of glass with a diamond-cut

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line across the centre. The object that it is desired to measure is set with one point exactly against the diamond-cut line, which, of course, will appear in the field, and the reading of the stage divisions taken. The stage is then slowly moved along by means of the milled head until the other edge of the specimen to be measured is exactly touching the line. The readings of the stage divisions are again taken, and by subtracting one from the other the measurement will be ascertained. For quick work, and without extraneous appliances, this is fairly accurate, and largely used.

2. *Camera Lucida and Stage Micrometer.*—A stage micrometer usually consists of a number of lines photographed, or ruled with a diamond on a slip of glass to the scale of $\frac{1}{100}$th or $\frac{1}{1000}$th part of an inch, or the $\frac{1}{10}$th and $\frac{1}{100}$th of a millimetre. This is put on the stage and focused like an ordinary object. The camera lucida is then fixed to the eyepiece, and the micrometer lines are projected on to a piece of paper in the same way as when drawing an object explained on page 112. The lines so projected are then measured, and supposing the lines of the micrometer, which are $\frac{1}{100}$th of an inch apart, appear when drawn on the paper 1 inch apart, it is at once known that the magnifying power in use is 100 diameters. The object may be measured in the same manner. Measurements should be taken about the centre of the field, and not towards the edge, especially with high powers, as, owing to curvature of the field, the outer edges appear more highly magnified than the centre.

3. *The Eyepiece Micrometer and Stage Micrometer.*—The stage micrometer, as previously described, is placed on the stage, and a somewhat similar micrometer is put into the eyepiece. This latter is generally divided into hundredths of an inch, but no exact value is needful so long as the lines are equidistant. On focusing the stage micrometer the two sets of lines will appear in the field at once. It is now desirable to ascertain how many divisions of the eyepiece micrometer are included between one of the spaces—that

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is, $\frac{1}{100}$ of an inch—of the stage micrometer. Perhaps it will be found that there will be several lines of the eyepiece micrometer and a fraction in that space, and in order that this fraction may be obviated the draw-tube should be slightly pulled out, which will give, of course, an increased amplification, until a certain number of the lines on the eyepiece micrometer are exactly equal to a division or divisions on the stage micrometer. We will imagine that the number of eyepiece micrometer lines that fill $\frac{1}{100}$ of an inch of the stage micrometer is five. The stage micrometer is now removed, and the object to be measured replaces it. The lines of the eyepiece micrometer will still be seen in the field, and bearing in mind that five of these lines equal $\frac{1}{100}$ of an inch, any part of the object can at once be measured. It must be remembered, however, that with every objective an estimation of the value of the eyepiece micrometer is necessary.

To give greater facility and accuracy, a form of eyepiece micrometer is used, devised by Jackson, which is fitted in a frame, and by means of a micrometer screw traverses the object. If there be no mechanical stage to the instrument it is very difficult to set a special part of the object against the micrometer for measurement, especially with high powers. This form of micrometer surmounts this difficulty. The ordinary eyepiece micrometers necessitate no alteration to ordinary eyepieces, but the Jackson form requires that the outer tube of the eyepiece shall be cut to receive the carrier for the micrometer. Fig. 45 shows an eyepiece with the Jackson micrometer, *m*, in position.

There is yet another form of eyepiece micrometer, called the Ramsden screw micrometer, which consists of an eyepiece containing two webs or wires, one fixed, the other travelling by means of a screw having 100 threads to the inch. The milled head of this screw is divided into 100 parts. Across the field are very small equidistant V-shaped teeth, the interval between each of which corresponds to one complete revolution of the milled head. The value of these teeth is

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taken against the stage micrometer, and the object placed on the stage. One edge of the object is then brought against the fixed wire, and the travelling wire moved to the other part that it is desired to gauge. By then counting the number of intervening teeth, and reading the fraction on the milled head, it can at once be ascertained what magnifying power is used. This is considered the most accurate and precise method of working, but it is an expensive piece of apparatus, and with care one of the previous methods named will be, as a rule, sufficient.

Persons having abnormal vision are likely to make errors in measuring. To obviate this, a cap carrying a lens that

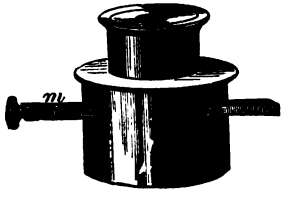

FIG. 45. — JACKSON MICROMETER FITTED TO EYEPIECE.

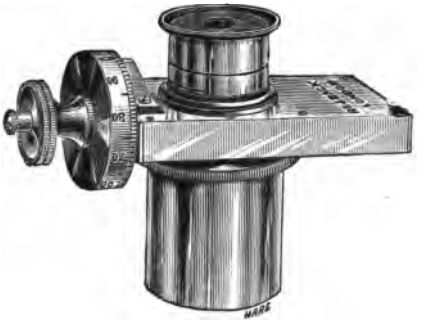

FIG. 46.—RAMSDEN SCREW MICROMETER.

will correct the abnormality should be placed over the top of the eyepiece, as described on page 78, while measurements are being taken.

Troughs, Live-cages, Stage Forceps, etc.

Troughs.—These are made of various materials, including glass, vulcanite, brass, etc., and are used in the examination of infusoria and animalculæ alive under the microscope. The essentials of a trough are that a medium power, say ½ inch at least, can be used, that it may be easily cleaned, and that if broken it can be repaired. The ordinary

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commercial glass troughs unfortunately do not meet these requirements. They are difficult to clean, they are invariably hard to mend when broken, and they very often leak when water is put in. The one that we have found most serviceable is the Botterill's trough, as shown in Fig. 47, which consists of two vulcanite plates between which are placed slips of glass, which are separated by an india-rubber band, small bolts and screws passing through the whole to hold them together, This is not an ideal trough, but it certainly answers its purpose as well as any at present made.

Live-cages.—These are not used so largely for water objects as for insects, etc. They consist of a brass plate

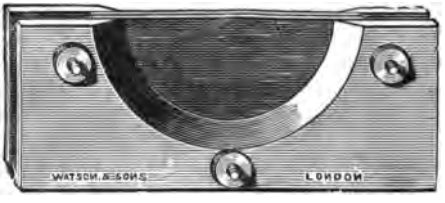

FIG. 47.—BOTTERILL'S TROUGH.

having a glass base-plate, over which a cap slides, having a very thin cover-glass. The subject to be viewed is placed between these two glasses and held firmly by compression. The best form is that designed by Mr. Rousselet, shown in Fig. 48, with which a condenser may be used conveniently. It is also so arranged that even if a specimen be fixed at the extreme edge of the glass plate, there is room for an objective to work on it. The ordinary live-cages are usually provided with a cover-glass too small in diameter for this to be done. A very good plan is often adopted by amateurs for viewing live objects as follows: A square, flat piece of glass is obtained, and on this an india-rubber ring is laid, into which the animalculæ can be placed; a thin piece of glass is now put over the top of the india-rubber ring, and this really makes a very serviceable trough.

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Rousselet's Compressor.—Mr. Rousselet, the designer of the live-cage previously mentioned, is also the originator of the most efficient compressor at present obtainable. It is shown in Fig. 49. The upper arm has cemented to its

FIG. 48.—ROUSSELET'S LIVE-CAGE.

under side a portion of a circle of thin cover-glass, which enables high-power objectives to be employed, and the disc of glass in the base-plate is not too thick to prevent the employment of condensers of large aperture. The compression is quite parallel in action, being effected by turning a milled head at the top of a drum containing a spring, which causes the upper plate to rise when the milled head is released. The cut-off top of the cover-glass permits of different media being inserted while the specimen is under examination, and the arm can be turned aside when desired for cleaning, etc.

FIG. 49.—ROUSSELET'S COMPRESSORIUM.

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Forceps.—Stage forceps are used to hold unmounted specimens in the field of view while they are examined, there being a fitting on the forceps to go into a hole provided in the limb or the stage of the instrument.

There are in existence many modifications of the apparatus described in the foregoing pages, the adoption or rejection of which must be left to the suggestions which will be naturally derived from practical experience. But the forms of apparatus most commonly worked with, and those whose merits particularly commend them to the writer's judgment, have been described.

Eye-shade for Monocular Microscope.

It is recommended when working with a monocular microscope that the eye not actually employed should

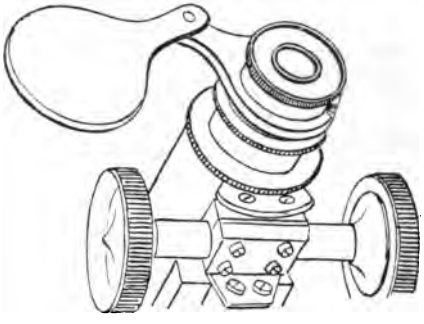

FIG. 50.

remain open. Many workers experience no difficulty in doing this, but others are quite unable to succeed. For such the eye-shade shown in the accompanying figure (Fig. 50) will be found very advantageous. It is made of vulcanite, and consists of two pieces jointed in the middle; one side is bored out to the outer diameter of the draw-tube, over which it slides, and the other portion is just a plain piece of sheet vulcanite which obscures light from the disengaged eye. It was introduced by C. Baker.

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HINTS REGARDING THE CARE OF THE MICROSCOPE.

Cleanliness is a most important feature in microscopical work. Never allow dust to accumulate upon the microscope, for it soon finds its way between the fittings, and causes mechanical screws to work with backlash. When not in use, the instrument should always be placed in its case or under a glass shade.

Dusting.—For dusting the microscope, a camel-hair brush should be used in the first place; by the aid of this, dust can be removed from niches and crevices with great ease. For wiping over the stand, also for cleaning eyepiece lenses, the fronts of objectives, and other optical work, the writer has always employed handkerchiefs that are made of a mixture of silk and cotton. These should be washed out two or three times until they are soft and free from dust.

Adjusting a Microscope.—It is not to be recommended that other than microscope makers should take the instruments to pieces; but it is often necessary, where a person resides abroad, that he should be able to adjust his own microscope. It is difficult to give definite advice, because the fittings vary considerably in every make of microscope. If the rack-work of the coarse adjustment or sub-stage develop loss of time, it is more often than not due to the bearings clutching on account of the presence of dust, or to their becoming dry. The way to adjust them is as follows: Rack the body up as far as it will go, and mark lines with a pen and ink on the pinion stem and the body of the microscope to correspond with one another. The object of this is to ensure the replacement of the body so that the rack engages the correct leaf of the pinion, and it is here presumed that a Jackson model microscope is used, and that it has a stop-pin to the rack, which prevents the body being removed from its fitting. Now remove the cock-piece, which holds the pinion in position, and take away

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the pinion itself, holding the body meantime, or it will run down on to the stage.

Remove the body from its fitting, wipe both bearings and the rack thoroughly with paraffin-oil and a clean rag, then dry them with another cloth. Now drop on, at most, two drops of watchmakers' oil on each side of the bearing fittings attached to the body, and replace the tube in its fittings. It should then be moved up and down until the motion is quite free, and if there are adjusting screws, they should be so set that there shall not be any shake in the fitting of the body, but that it may just, and only just, move in the bearings with its own weight when the instrument is set vertically. Carefully wipe the pinion leaves out, and then, after setting the ink-marks in correspondence again, the pinion may be attached. This usually has adjusting screws to the cockpiece, which push it closer to, or allow it to remain farther from, the rack; these should be so set as to give a soft movement. It is useless to attempt this procedure with a microscope that never has worked well, but where an instrument has, after use, become unsatisfactory in the mechanical parts, it generally is found to answer. Practically the same treatment is applicable to the mechanical movements of the stage, but very great care is essential, lest either of the plates becomes bent, an accident that is more easy of occurrence than would be deemed likely.

Objectives.—It is unwise for unskilled persons to unscrew parts of microscopic objectives; they are frequently deranged by this means. If at any time it should, from some cause or another, be necessary to unscrew them, an ink-mark or small scratch should be made on each combination, so that when put together again they can be screwed up in the same positions as before. In many objectives it will be noted that three or four little spots are engraved in a line down the mount for the above purpose.

After using an oil immersion objective, the oil must be carefully removed from the front lens by wiping with

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the handkerchief. Undue pressure must not be used, but it must be thoroughly cleaned. If oil should become dried on the front lens at any time, it will be best to place some fresh immersion-oil over it; it should then be allowed to stand in a place free from dust for about an hour, when the whole may be cleaned off together.

Travelling.—When travelling with a microscope it is always well to pack the instrument round with tissue-paper, so that it cannot shake in its case. Screws frequently become loosened, and in some instances broken, and movements disordered, by severe shaking while in transit.

For a more exhaustive treatment of the subject the following text-books are recommended:

Carpenter's 'The Microscope and its Revelations,' edited by Dr. Dallinger.

'The Microscope,' by Dr. Henri van Heurck.

'Photo-micrography,' by Dr. Spitta.

'Photo-micrography,' by Mr. Andrew Pringle.

And as periodicals the best are: *Knowledge*, published by Messrs. Witherby and Co., 326, High Holborn, W.C.; *Science Gossip*, published at 110, Strand, W.C.; the *Journal of the Royal Microscopical Society*, published bi-monthly, and the *Journal of the Quekett Club*, published twice a year, both at 20, Hanover Square, W.C.

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APPENDIX.

A short note concerning the influence of diffraction on the resolving power of microscopical objectives, and on the apparent colour of microscopical objects.

By Dr. G. Johnstone Stoney, F.R.S.

If we look from a distance at a flame through a thin feather or other uniformly ruled grating we see the flame, and around or on either side of it a number of lateral coloured images, which are wider and usually fainter the farther out that they lie. We thus learn that the light which passes through the grating becomes both a direct beam and a number of lateral, or diffracted beams as they are called. The proportions in which the light which passes the grating is distributed between the central beam and the several diffracted beams depends upon the ratio of the widths of the openings to the widths of the bars of the grating, as well as upon such particulars as whether each opening is a mere hole and each bar a mere obstruction, or whether they are occupied by material which acts on the light, especially if it act like a prism. It rarely happens that this distribution does not perceptibly differ for light of different wave-lengths. The direct beam consists of light in very nearly the same state as if it had passed through a simple opening of the size of the grating, except that it is fainter—usually fainter in some colours than in others.

Accordingly, if the eye when looking at the grating, or if the object-lens of a telescope, were to receive only this

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central beam wherewith to form an image of the grating, the image would be almost identical with that which would be furnished by light coming through an opening covered by tinted glass, and no trace of the ruling would be seen in it. In order to see the ruling, the telescope lens must be able to catch and forward to its focus other rays which have passed through the grating than those of the central beam. The more of the lateral beams which it can transmit and combine at its focus with the light of the central beam (where they will by interference strengthen some parts of the image formed by the central beam, and enfeeble others, thus *introducing* detail), the more nearly will the image it forms resemble the actual grating in detail, and in freedom from false colour. If it succeeds in catching, along with the central beam, even some small portion of the nearest of the diffraction beams, the image will exhibit lines, and the proper number of lines, though it will not present correctly such minuter detail as the widths of the lines and of the spaces between them.

Cases exactly analogous to this occur with the microscope. When an object covered with dots, such as *Pleurosigma angulatum*, has been focused upon the stage, and is resolved, the diffraction beams may be clearly seen upon the back lens of the objective by removing the eyepiece and looking down the tube. With this diatom there will then be seen the central beam, and portions of the nearest of the lateral beams, six in number. A rather small cone of illumination is best to show them conspicuously if white light be used, and they can be seen with larger cones of illumination and very sharply defined if monochromatic green light, produced by prisms, be employed.

The markings on the *Pleurosigma angulatum* are spaced in each row at intervals which have been measured, and found to be equal to wave-lengths of red light. With so close a ruling the lateral beams are much diffracted or bent aside, and dry objectives can only take in the central beam and a portion of each of the nearest diffracted beams. This

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enables us to see with such an objective the markings correctly so far as concerns their number and positions, but any further detail is not correctly presented. Immersion objectives can transmit nearly the *whole* of the six nearest lateral beams, which are those that would produce spectra of the first order. We now see some detail: the dots appear hexagonal, and are separated from one another by walls which are thin, and which look like a honeycomb. This is the first and the only step we can take towards learning what the actual detail upon this diatom is, since no objective is competent to supply to the image the second or subsequent diffraction beams; inasmuch as no immersion fluid can shorten the waves of visible light so much as would enable the object to emit and the lens to receive these further diffraction beams.

The unequal distribution of *colour* between the several beams is strikingly exhibited by the diatom known as *Actinocyclus Ralfsii*. The phenomenon may be conveniently examined through a half-inch apochromatic, over which is mounted an iris diaphragm—an adjunct which is useful for many purposes. Select a frustule which is blue when this upper diaphragm is partly closed. Remove the eyepiece, close the lower, and open the upper diaphragm. Then, on looking down the tube, it will be seen that most of the red is located in a ring of first lateral beams, with, of course, an equal defect of red in the central beam. Hence the blue colour seen when the image is formed by the central beam only. Now place a small central stop (which may be cut out of card) over the back lens of the objective, open the upper, and partially close the lower diaphragm. The image is then formed by the ring of lateral beams only, and will be found to be preponderatingly red.

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PART II.

INTRODUCTION.

In publishing methods of preparing, staining, hardening, and mounting microscopic objects, I have adopted the system employed in my classes for some years past; that is, each separate stage of procedure is arranged in successive lessons or chapters. A subject such as this cannot be so lucidly described in writing as by demonstration, but it has been my aim to make it as clear as possible, so that if the instructions are carefully followed and practised, successful permanent work can be performed; but it is only by most scrupulous care and constant practice that any degree of success in this work can be attained.

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LESSON NO. 1.

HARDENING AND PRESERVING ANIMAL TISSUES AND LISTS OF MATERIALS.

LIST OF TISSUES AND ORGANS, AND THE MOST SUITABLE HARDENING, STAINING, AND MOUNTING REAGENTS.

TISSUES.	HARDENING REAGENT.	STAINING FLUID.	MOUNTING MEDIUM.	ANIMAL.
Blood, human.	Dry on slide.	Eosin.	C. Balsam.	
Do., amphibia.	Do.	Eosin and Methyl green.	Do.	Frog.
Epithelium.	2 % Bichromate potash.	Picrocarmine.	Farrant's.	Do.
Endothelium.	Nil.	Silver nitrate.	Farrant's or C. Balsam.	Rabbit.
White fibrous tissue.	Alcohol.	Nil.	Farrant's or glycerine jelly.	Tail of rat.
Yellow elastic tissue.	Chromic acid and spirit.	Nil.	Do., do.	Lig. nuchæ of ox.
Adipose tissue.	Methylated spirit.	Hæmatoxylin.	Do., do.	Any animal.
Tendon.	Do., do.	Do.	Do., do.	Sheep.
Adenoid tissue.	Müller's fluid.	Do., and eosin.	C. Balsam.	Cat.
Cartilage.	Chromic acid and spirit.	Do., do.	Do.	Do.
Bone.	Chromic and nitric acid.	Picrocarmine.	Farrant's.	Do.
Do., developing.	Do., do.	Hæmatoxylin and eosin.	C. Balsam.	Kitten.
Marrow.	Methylated spirit.	Hæmatoxylin.	Do.	Guinea-pig or cat.
Muscle, striated.	2 % Bichromate potash.	Do., and eosin.	Do.	Cat.
Do., non-striated.	Chromic acid and spirit.	Hæmatoxylin.	Balsam or Farrant's.	Colon of rabbit.
Nerve-fibres.	Osmic acid	Osmic acid.	Farrant's.	Sciatic of frog.
Do., trunk.	Chromic acid and spirit.	Hæmatoxylin and eosin.	C. Balsam.	Do. of cat.
Bloodvessels.	Do., do.	Do., do.	Do.	Cat.
Lymphatic glands.	Müller's fluid.	Do., do.	Do.	Do.
Tonsil.	Methylated spirit.	Do., do.	Do.	Do.
Thymus gland.	Müller's fluid.	Do., do.	Do.	Human fœtus or calf.

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LIST OF TISSUES AND ORGANS, AND THE MOST SUITABLE HARDENING, STAINING, AND MOUNTING REAGENTS—*continued.*

TISSUES.	HARDENING REAGENT.	STAINING FLUID.	MOUNTING MEDIUM.	ANIMAL.
Skin.	Methylated spirit.	Hæmatoxylin and eosin.	C. Balsam.	Human palm of hand.
Nail.	Do., do.	Do., do.	Do.	Human fœtus.
Scalp.	Do., do.	Do., do.	Do.	Do.
Heart muscle.	Chromic acid and spirit.	Do., do.	Do.	Cat.
Trachea.	Do., do.	Do., do.	Do.	Do.
Lung.	Do., do.	Do., do.	Do.	Do.
Tooth.	Chromic and nitric acid.	Picrocarmine.	Farrant's.	Do.
Do., developing.	Do., do.	Do.	Do.	Kitten about two months old.
Tongue.	Chromic acid and spirit.	Hæmatoxylin and eosin.	C. Balsam.	Cat.
Œsophagus.	Do., do.	Do., do.	Do.	Do.
Stomach, cardiac end.	Absolute alcohol.	Soluble aniline blue.	Do.	Do.
Do., pyloric end.	Do., do.	Hæmatoxylin and eosin.	Do.	Do.
Small intestine.	Chromic acid and spirit.	Do., do.	Do.	Do.
Large intestine.	Do., do.	Do., do.	Do.	Do.
Liver.	2 % Bichromate potash.	Do., do.	Do.	Do.
Pancreas.	Absolute Alcohol.	Do., do.	Do.	Do.
Salivary glands.	Do., do.	Do., do.	Do.	Do.
Spleen.	2 % Bichromate potash.	Do., do.	Do.	Do.
Supra-renal glands.	Methylated spirit.	Do., do.	Do.	Guinea-pig or cat.
Thyroid glands.	Do., do.	Do., do.	Do.	Cat.
Kidney.	2 % Bichromate potash.	Do., do.	Do.	Do.
Ureter.	Chromic acid and spirit.	Do., do.	Do.	Do.
Testicle.	Methylated spirit.	Do., do.	Do.	Do.

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LIST OF TISSUES AND ORGANS, AND THE MOST SUITABLE HARDENING, STAINING, AND MOUNTING REAGENTS—*continued.*

TISSUES.	HARDENING REAGENT.	STAINING FLUID.	MOUNTING MEDIUM.	ANIMAL.
Vas deferens.	Methylated spirit.	Hæmatoxylin and eosin.	C. Balsam.	Cat.
Epididymis.	Do., do.	Do., do.	Do.	Do.
Prostate.	Do., do.	Do., do.	Do.	Do.
Ovary.	Do., do.	Do., do.	Do.	Do.
Fallopian-tube.	Do., do.	Do., do.	Do.	Do.
Uterus.	Do., do.	Do., do.	Do.	Do.
Mammary gland.	Do., do.	Picrocarmine.	Farrant's or C. Balsam.	Do.
Spinal cord.	2 % Bichromate ammonium.	Aniline blue black.	C. Balsam.	Do.
Medulla oblongata.	Do., do.	Do., do.	Do.	Do.
Pons Varolii.	Do., do.	Do., do.	Do.	Do.
Cerebellum.	Do., do.	Do., do.	Do.	Do.
Cerebrum.	Do., do.	Do., do.	Do.	Do.
Eyelid.	Methylated spirit.	Hæmatoxylin and eosin.	Do.	Do. or human fœtus.
Cornea.	Müller's Fluid.	Do., do.	Do.	Do. or sheep.
Choroid.	Do., do.	Do., do.	Do.	Sheep.
Crystalline lens.	2 % Bichromate potash.	Picrocarmine.	Farrant's.	Cat.
Retina.	Müller's Fluid.	Hæmatoxylin and eosin.	C. Balsam.	Ox.
Sclerotic.	Do., do.	Do., do.	Do.	Do.
Optic nerve.	Do., do.	Do., do.	Do.	Do.
Olfactory mucous membrane.	Do., do.	Do., do.	Do.	Rat.
Internal ear. Cochlea.	Do., do., and decalcify.	Carmine in bulk.	Do.	Guinea-pig.

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LIST OF BOTANICAL SPECIMENS, AND THE MOST SUITABLE PRESERVING, STAINING, AND MOUNTING MEDIA.

SPECIMEN.	PRESERVING REAGENT.	STAINING FLUID.	MOUNTING MEDIUM.
Stems, young.	Methylated spirit.	Hæmatoxylin.	C. Balsam.
Do., older.	Do., do.	Carmine and acid green.	Do.
Leaves.	Do., do.	Hæmatoxylin.	Do.
Ovaries.	Do., do.	Do.	Do.
Anthers.	Do., do.	Borax carmine.	Do.
Epidermis for stomata.	Macerate in water.	Methyl aniline.	Glycerine jelly.
Fibro-vascular tissues.	Do., do.	Acid aniline green.	Do.
Yeast.	Camphor-water.	Unstained.	Camphor-water.
Green algæ.	Acetate of copper solution.	Do.	Acetate of copper solution.
Red algæ.	Dilute methylated spirit.	Do.	Glycerine jelly.
Protococcus.	Acetate of copper solution.	Do.	Acetate of copper solution.
Volvox.	Do., do.	Do.	Do., do.
Desmids.	Do., do.	Do.	Do., do.
Raphides.	Macerate in water.	Do.	C. Balsam.
Starches.	Methylated spirit.	Do.	Glycerine jelly.
Fertile branch of chara.	Do., do.	Do.	Camphor-water.
Antheridia and archegonia of mosses.	Do., do.	Do.	C. Balsam.

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LESSON I.

HARDENING AND PRESERVING ANIMAL TISSUES AND ORGANS FOR MICROSCOPICAL EXAMINATIONS.

Fresh untreated tissues are unsuited for microscopical purposes, but it is sometimes advisable to observe the appearance of some specimens, such as muscle-fibres, tendon, connective tissues, and nerve-fibres, while fresh. When this is desired, the tissue must be examined in certain fluids called normal fluids that will alter its character as little as possible. Those generally used are: (1) blood serum; (2) the aqueous humour from a fresh eye; and (3) normal or ¾ per cent. salt solution. The two former are difficult to obtain, but the latter can be made at any time, and it will answer for most purposes. Place a small piece of the tissue on a slide, add a drop or two of salt solution, take two needles fixed in holders and carefully separate the fibres from each other; this process is called teasing. When sufficiently teased, apply a cover-glass and examine. You may now wish to irrigate with some staining reagent; if so, place a few drops of the stain at one edge of the cover-glass, and apply a piece of blotting-paper to the other side; this will absorb the salt solution, and the staining fluid will follow and take its place around the tissue; the slide may then be placed under the microscope, and the action of the reagent observed.

These specimens cannot, as a rule, be kept. For permanent preparations the tissues or organs must be hardened. This is accomplished by subjecting them to the action of certain hardening or fixing solutions. The following are most commonly used:

Absolute Alcohol.—Suitable for stomach, pancreas, and salivary glands. These organs must be perfectly fresh,

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and they should be cut into small pieces, so that the alcohol may penetrate as quickly as possible.

Change the alcohol every day for the first three days. The hardening is usually complete in a week.

Chromic Acid and Spirit.—Chromic acid $\frac{1}{6}$ per cent., watery solution 2 parts, and methylated spirit 1 part. This reagent hardens in about ten days. Then transfer to methylated spirit, which should be changed every day until no colour comes away from the tissues. It is suitable for cartilage, nerve-trunks, heart, lips, blood-vessels, trachea, lung, tongue, bladder, ureter, intestines, and œsophagus.

Potassium Bichromate.—Make a 2 per cent. watery solution. This will harden in about three weeks. Then transfer to methylated spirit, and change the spirit every day until no colour comes away from the tissues. It is suitable for muscle, spleen, liver, and kidney.

Ammonium Bichromate.—Make a 2 per cent. watery solution. It hardens in from three to four weeks. Then transfer to methylated spirit, and change every day until no colour comes away from the tissues. It is suitable for spinal cords, medulla, pons Varolii, cerebellum, and cerebrum.

Müller's Fluid.—Bichromate of potash 30 grains, sulphate of soda 15 grains, distilled water $3\frac{1}{2}$ ounces. It hardens in from three to five weeks. Then transfer to methylated spirit, and change every day until no colour comes away from the tissues. Suitable for lymphatic glands, eyeballs, retina, and thymus gland.

Methylated Spirit.—May be used universally, if preferred, but it has a tendency to shrink some tissues too much. It hardens in about ten days. Change the spirit every twenty-four hours for the first three days. Suitable for skin, scalp, testicle, penis, prostate gland, vas deferens, epididymis, ovary, uterus, Fallopian tubes, placenta, mammary gland, supra-renal glands, tonsils, and all injected organs.

Decalcifying Solution.—For bones. Make a $\frac{1}{6}$ per cent.

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watery solution of chromic acid, and for every ounce add 5 drops of nitric acid. This fluid will soften the femur of a dog in about three weeks; larger bones will take longer. Change the fluid several times, and test its action by running a needle through the thickest part of the bone. If it goes through easily, the bone is soft enough; if not, continue the softening process a little longer. When soft enough transfer to water, and soak for an hour or two; then pour off the water and add a 10 per cent. solution of bicarbonate of soda, and soak for twelve hours to remove all trace of acid. Wash again in water, and place in methylated spirit until required. Bones and teeth should always be softened in a large quantity of the decalcifying solution.

Olfactory Region.—Divide with a saw the head of a freshly killed rabit or guinea-pig longitudinally, and parallel with the nasal septum. Cut out the septum so as to expose the olfactory region, which is recognised by its brown colour. Dissect out a portion including some of the turbinated bones. Harden this in Müller's fluid for three or four days. Then transfer to chromic and nitric acid decalcifying solution, and soak until the bones are quite soft. Wash well in water to remove all trace of acid, and complete the hardening in methylated spirit.

Cochlea.—Dissect out the internal ear of a freshly killed young guinea-pig, open bulla with bone-forceps, when a conical elevation, the cochlea, will be seen. Remove as much of the surrounding bone as possible, and place the cochlea in Müller's fluid for two weeks to harden the delicate nervous tissues. Then transfer to chromic and nitric acid decalcifying solution, and soak until the bone is soft. Place in weak spirit for a day or two, and then transfer to strong methylated spirit.

Corrosive Sublimate.—Tissues may be fixed very quickly in corrosive sublimate. Make a saturated solution in 5 per cent. glacial acetic acid. The specimens should be removed from the solution as soon as they are fixed,

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directly they become opaque throughout. Then wash in repeated changes of 70 per cent. alcohol to which a little tincture of iodine has been added. This process will fix tissues in a few minutes.

Picric Acid.—Make a saturated solution in water. This solution will fix small pieces of tissue in a few minutes; larger specimens will require from three to six hours' immersion. Then wash out the picric acid with repeated changes of spirit. Water must not be used, as it is hurtful to the tissues that have been prepared by this method. For the same reasons, during all subsequent stages of treatment, water should be avoided, and the staining should be carried out in alcoholic solutions.

Formaldehyde.—This may be used universally if desired. It is sold commercially as 'formal' in a 40 per cent. solution. This must be reduced by the addition of water to a 2 or 4 per cent. solution. It is specially useful for hardening nervous tissues and for eyes; the latter are completely hardened in 24 hours.

When in great haste, tissues may instantly be fixed in boiling water. Boil some water in a test-tube, then drop in small pieces of the tissue, and boil again for a few seconds. The specimen may then be placed at once in gum and syrup, and when penetrated, freeze, and make the sections. This method should only be used when a section is urgently wanted.

General Directions for Hardening Tissues.

1. Always use fresh tissues.
2. Cut the organs into small pieces with a sharp knife.
3. Never wash a specimen in water; when it is necessary to remove any matter, allow some normal salt solution to flow over the surface of the tissue, or wash in some of the hardening reagent you are going to use.
4. All specimens should be hardened in a large quantity of the reagent; too many pieces should not be put into the bottle, and they should be kept in a cool place.

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5. In all cases the hardening process must be completed in spirit.
6. Label the bottles, stating the contents, the hardening fluid used, and when changed. Strict attention to these details is necessary for successful histological preparations, for if the hardening is neglected good sections cannot be made.

LESSON II.

EMBEDDING TISSUES AND SECTION-CUTTING.

To Cut Sections with a Razor by Hand.—Take the tissue between the thumb and forefinger of the left hand. Hold the finger horizontally, so that its upper surface may form a rest for the razor to slide on. Take the razor, hold it firmly in the hand, keep the handle in a line with the blade, and draw it through the tissue from heel to tip towards yourself. While cutting, keep the razor well wetted with dilute methylated spirit, and as the sections are cut place them in a saucer of dilute methylated spirit.

Embedding in Paraffin Wax and Lard.—Melt together by the aid of gentle heat four parts of solid paraffin and one part of lard. A quantity of this may be made and kept ready for use at any time. Melt the paraffin mass over a water-bath. Take the specimen and dry it between the folds of a cloth to remove the spirit, so that the paraffin may adhere to its surfaces, place it in a pill-box in the desired position, and pour in enough melted paraffin to cover it, then set aside to cool. When quite cold, break away the pill-box and cut sections from the embedded mass with a sharp razor. When a number of specimens are embedded, and it is desired to keep them for some time, they should be preserved in a jar of methylated spirit.

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To Infiltrate a Tissue with Paraffin.—Dehydrate the specimen in absolute alcohol for several hours, then transfer to chloroform or xylol, in which it must remain until perfectly saturated. When clear, place in a bath of melted paraffin of 45° C. (melting-point), and keep it at this temperature for several hours, so that the paraffin may penetrate to the middle of the tissue. Then remove it from the paraffin and put it into a small pill-box, pour in enough paraffin to fill the box, and as the paraffin cools, add a little more to make up the skrinkage and set aside to cool. When cold, place in water for a few minutes; this will soften the paper, and facilitate the removal of the pill-box. You will now have a cylinder of paraffin with the specimen firmly fixed in its centre, and, if desired, the paraffin may be pared away from the sides until a square block is obtained. The sections may now be made by hand with a razor, or the block can be fixed to a microtome with a little melted paraffin. The sections must be placed in turpentine to remove the paraffin, then in absolute alcohol to remove the turpentine, and, finally, in distilled water to remove the alcohol; they may then be stained. Sometimes it is desirable to stain the tissue in bulk before it is embedded. In this case the sections need only go into turpentine or benzole to wash away the paraffin; they may then be mounted in Canada balsam.

The above process requires an embedding bath. This is usually an expensive affair, but one that will answer all ordinary purposes can easily be made.

Get a small potato-steamer, and cut a hole in the lower vessel to admit a spirit or small paraffin lamp. Get a tinsmith to cut out a circular plate of tin to fit into the upper vessel, in which some holes must be cut to take the test-tubes, and to the sides of the vessel four small pieces of tin, bent at right angles, must be soldered to support the tin plate. A piece of tin must also be soldered over the perforated bottom of the vessel, so that it will hold water. When the alterations are complete, place a layer of cotton-

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wool or a piece of felt on the bottom of the steamer, to protect the test-tubes from breakage; half fill with water, add a thermometer, light the lamp, and on the desired temperature being attained, put some paraffin in the test-tubes, place them in the steamer, and when the paraffin has melted add the specimens.

After use dry the apparatus so that rust may not set in. If this is attended to it will last for years.

When a proper embedding bath cannot be obtained,

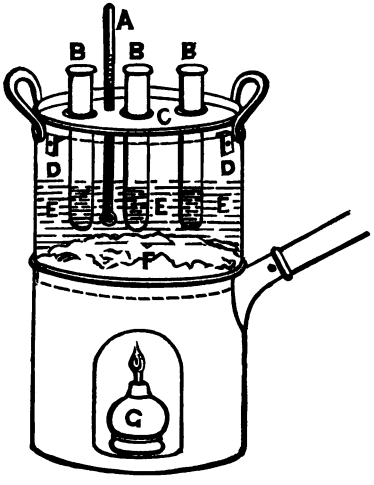

FIG. 51.—POTATO-STEAMER CONVERTED INTO AN EMBEDDING BATH.

A, Thermometer; B, Test-tubes; C, Disc of tin; D, Tin supports; E, Water; F, Cotton-wool; G, Spirit or small paraffin lamp.

tissues may be infiltrated with paraffin in the following way: Dehydrate the specimen in absolute alcohol; then place in a quantity of chloroform or benzole, ten or twelve times the bulk of the tissue, until saturated; add small pieces of paraffin until no more will dissolve, and set aside for several hours. Apply gentle heat to drive off the solvent and melt the paraffin, after which the tissue can be removed and embedded in a pill-box of paraffin of the desired melting-point.

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Cole's Microtome and Embedding in Carrot.—When a number of sections are wanted, or when a complete section of an organ is desired, a microtome should be used. A very good and simple instrument can be obtained from Messrs. Watson and Sons, 313, High Holborn. Screw the microtome firmly to the table, and with the brass tube supplied with the microtome punch out a cylinder of carrot to fit into the well of the microtome. Cut this in half longitudinally, and scoop out enough space in one half of the carrot to take the specimen; then place the other half of carrot in position, and make sure that the specimen is held firmly between them, but it must not be crushed. Now put the cylinder of carrot and specimen into the well

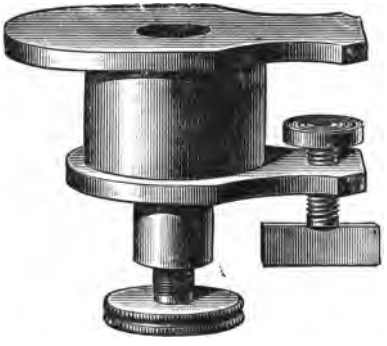

FIG. 52.—COLE'S PATTERN MICROTOME.

of the microtome and commence cutting the sections. A good razor will do, but it is better to use the knife which Messrs. Watson supply with the microtome. While cutting, keep the knife and plate of the microtome well wetted with dilute methylated spirit, and as the sections are cut place them in a saucer of dilute spirit. A number of sections may be cut and preserved in methylated spirit until required.

When a specimen has a very irregular outline, it cannot be successfully embedded in carrot. Paraffin should then be used. Place the tissue in the well of the microtome in the desired position, pour in enough melted paraffin to cover it, and when cold cut the sections.

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Freezing Microtome.—Cathcart's is the most simple and cheapest freezing microtome, and it can be obtained from any optician.

(1) Cut a slice of the specimen about $\frac{1}{8}$-inch thick, in the direction you wish to make the section.

(2) Place in water for an hour to remove the alcohol.

(3) Transfer to a mixture of gum-water 5 parts, saturated

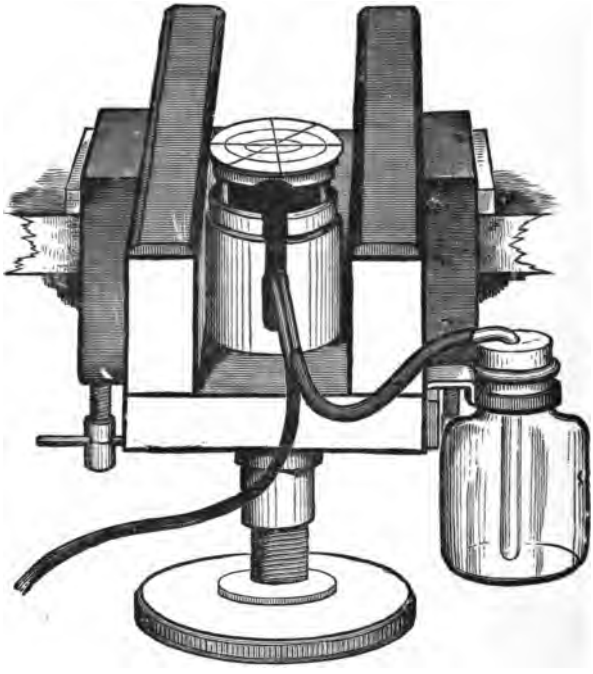

FIG. 53.—CATHCART'S MICROTOME.

watery solution of loaf-sugar 3 parts, and allow it to soak in this for about twelve hours; or, if a few drops of carbolic acid are added to the mixture, tissues may remain in it for months without harm.

(4) Clamp the microtome to a table, fix the ether spray in its place, and fill the bottle with ether. Methylated ether, sp. gravity 720, will do.

(5) Put a little gum-water—not gum and syrup—on the

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zinc plate of the microtome, and place the tissue in it. Commence working the bellows, and as soon as all the gum has frozen add some more and freeze again, and so on until the tissue is completely covered and frozen into a solid mass.

(6) The best instrument for making the sections is the blade of a carpenter's plane. Hold it firmly in the right hand, and work the microtome screw under the machine with the left. Plane off the sections as quickly as possible. They should all collect on the plane iron. If they roll up or fly off, the tissue is frozen too hard, or there is not enough syrup in the gum. If the former is the case, allow the mass to thaw a little; if the latter, add some more syrup to the gum mixture, and soak the tissue again.

When the sections are cut, place them in a saucer of water, which must be changed several times until all trace of gum is removed. Water that has been boiled and allowed to cool will remove the gum sooner than cold water. When quite free from gum, the sections may be bottled up in methylated spirit until required for staining.

Embedding in Celloidin.—Dissolve Schering's celloidin in equal parts of absolute alcohol and ether until the solution is as thick as glycerine. Divide the solution into two parts, to one of which add an equal part of absolute alcohol and ether. Dehydrate the specimen in absolute alcohol for several hours, then transfer to the thinner solution of celloidin, and soak until perfectly saturated; place in the thick celloidin for about an hour, or until required. Take a cork and paint over one end a layer of celloidin, and let it dry; this will prevent air bubbles rising from the cork and lodging in the mass. Take the specimen from the celloidin and lay it on the cork, and let it stand for a minute or two, then add some more celloidin until the tissue is completely covered, and set aside, and when the mass has attained such a consistency that on touching it with the finger no impression will remain, place it in 50 per cent. alcohol for an hour or two to complete the hardening, or it

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may remain there until required. The embedded mass can now be placed between two pieces of carrot, and put into an ordinary microtome, and the sections made with a knife or razor, which must be well wetted with methylated spirit; or the embedded specimen can be removed from the cork, and, after soaking in water, it can be transferred to gum and syrup, and the sections made with a Cathcart freezing microtome. If it is desired to remove the celloidin from the sections, soak them in equal parts of absolute alcohol and ether. When all the celloidin is removed, transfer to distilled water, then into the stain. After staining, wash in distilled water, dehydrate, clear in clove oil, and mount in Canada balsam.

When it is not desirable to remove the celloidin from the sections, they should be stained in borax carmine or hæmatoxylin. The former stains celloidin, but the colour is removed by washing in acidulated alcohol. Hæmatoxylin only stains it slightly. All the aniline dyes stain it deeply; they should not be used.

Tissues are usually stained in bulk before they are infiltrated with celloidin. When so, the sections must be dehydrated in methylated spirit, cleared in oil of bergamot or origanum, and mounted in Canada balsam.

When desirable, sections infiltrated with celloidin may be mounted in Farrant's medium or glycerine jelly. Wash away all trace of alcohol with water, and mount in either of the above media in the ordinary way.

Celloidin is an excellent medium for infiltrating many specimens of both animal and vegetable subjects. The following are a few of these:

Flower-buds of lily, yucca, evening primrose, poppy, dandelion, and anthers; worms, leech, flukes, gills and organs of mussels, heads of frogs, newts, sponges, etc.

For flower-buds proceed as follows: Harden the bud in methylated spirit in the ordinary way. Then take a piece of fine silk or cotton and tie it round the centre of the bud to

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hold the parts together; now with a sharp knife cut off each end of the bud so that the celloidin may easily penetrate to the interior. Now place the specimen in equal parts of absolute alcohol and ether for at least twelve hours. Then transfer to the thin solution of celloidin, and soak until completely infiltrated. Remove and place in thick celloidin for about twelve hours. Take out of celloidin on the point of a needle, and hold exposed to the air for a few minutes, to dry the celloidin around the exterior of the bud. When dry, push gently off the needle into some methylated spirit, and soak for at least twelve hours to complete the hardening of celloidin. The specimen may then be embedded in carrot, and the sections may be cut in any ordinary well microtome. Worms must be cut up into pieces of about ¼ or ½ inch long; these are then dehydrated in equal parts of ether and alcohol, infiltrated with and embedded in celloidin, and then treated in exactly the same way as directed for flower-buds.

When a number of celloidin masses are prepared for future use, they must be preserved in a vessel of methylated spirit.

Embedding in Gelatine.—This method is very useful for hairs, cotton, silk, wool, and all such fibres. Take, for example, some human hairs about ½ an inch long, and make a bundle of them; tie them together either with a long hair or with some fine cotton. Place the bundle in warm water and soak for a few minutes. Now make up a strong solution of some clear transparent gelatine. Cox's is very good—say 1 ounce of gelatine to 6 of water. Transfer the bundle of hair to this, place in a warm water bath, and soak until the gelatine has penetrated all through the bundle. Remove from gelatine on the point of a needle, and allow the mass to cool; then place in methylated spirit for about twelve hours. The embedded mass may then be placed in a cylinder of carrot and transverse sections cut in the ordinary well microtome. The sections when cut are to be placed in strong spirit to dehydrate;

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they are then cleared in clove oil and mounted in C. Balsam.

Heads of frogs, newts, and many other specimens may be infiltrated and embedded in gelatine, but they must all be stained in bulk before they are infiltrated, because the sections must not come in contact with water in any form; moreover, if the sections were stained the gelatine would be coloured as well as the tissues.

The Rocking Microtome.—This machine is made by the Cambridge Scientific Instrument Company. It is only used

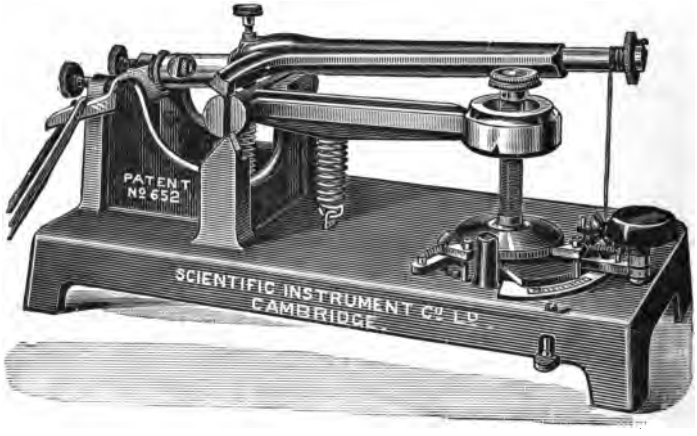

FIG. 54.—ROCKING MICROTOME.

for specimens infiltrated with paraffin, and it is automatic—that is to say, it can be set to cut sections of definite thickness, and every time the handle is pulled a section is cut, and the specimen is moved forward ready for another.

Infiltrate the tissue with paraffin in the ordinary way in a pill-box, and when the paraffin has set, remove the box and trim the paraffin into a rectangular block. Take care to keep the edges quite parallel, so that they may adhere together as the sections are cut and form a riband. The Cambridge Instrument Company make an apparatus for embedding, called embedding L's. If these are used, per-

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fectly rectangular blocks are formed ready for fixing to the brass cap at the end of the arm of the microtome, which is filled with paraffin; this should be warmed over a spirit-lamp, and the block containing the specimen is to be pressed against the melted paraffin until it adheres firmly.

LESSON III.

STAINING ANIMAL SECTIONS AND MOUNTING IN CANADA BALSAM.

All sections of organs and tissues should be stained with some colouring reagent, so that their structure may be made more apparent. Certain parts of the tissue have a special affinity for the dyes or stain; they therefore become more deeply tinted, and stand out clearly from the surrounding tissues.

The following staining reagents are the most useful:

Grenacher's Alcoholic Borax Carmine. — Carmine 3 grammes; borax 4 grammes; distilled water 100 c.c. Dissolve the borax in the water, add the carmine, and apply gentle heat until all is dissolved; then add 100 c.c. of 70 per cent. alcohol, filter, and keep in a stoppered bottle.

Staining Process.—(1) Place the section in distilled water to wash away the alcohol, then place a little of the carmine in a watch-glass, and immerse the section for from three to five minutes.

(2) Wash the section in methylated spirit.

(3) Take of methylated spirit 5 parts, and of hydrochloric acid 1 part, and mix them well together. A quantity of this acid solution may be made up and kept ready for use at any time.

Immerse the section in the above, and leave it to soak for about five to ten minutes, or, if overstained, until the desired tint is obtained. Sections of skin and scalp may be left until all colour is removed from the fibrous tissues; the

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glands, hair follicles, and Malpighian layer will then stand out clearly.

(4) Wash the section well in methylated spirit to remove all traces of the acid, then transfer to some perfectly clean and strong methylated spirit for from ten to fifteen minutes to dehydrate.

(5) Place some oil of cloves in a watch-glass, take the section from the spirit on a lifter, and carefully float it on to the surface of the oil, in which it must remain for about five minutes. This process is called clearing; the object of it is to remove the alcohol and to prepare the section for the balsam.

(6) Transfer the section to some filtered turpentine to wash away the oil of cloves, and mount it in Canada balsam. Sections may be mounted in Canada balsam direct from the oil of cloves, but it is better to wash in turpentine first, because if much oil is mixed with the balsam it will not dry; the oil also has a tendency to cause the balsam to turn a dark-yellow colour.

Ehrlich's Hæmatoxylin. — Hæmatoxylin 30 grains, absolute alcohol 3½ ounces, distilled water 3½ ounces, glycerine 3½ ounces, and ammonia alum 30 grains. Dissolve the hæmatoxylin in the alcohol and the alum in the water; mix the two solutions together, and add the glycerine and 3 drachms of glacial acetic acid. The mixture must now be left exposed to light for at least a month, then filter and keep in a stoppered bottle.

Staining Process.—(1) If the specimen has been hardened in any of the chromic solutions, place the section in a 1 per cent. watery solution of bicarbonate of soda for about five minutes, then wash well in distilled water. If it is a spirit preparation the soda will not be required, but all sections must be washed in distilled water before they go into hæmatoxylin stain.

(2) To a watch-glassful of distilled water add from 10 to 20 drops of the hæmatoxylin solution, and immerse the section for from ten to thirty minutes.

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(3) Wash in distilled water, then in ordinary tap water; the latter will fix the dye and cause the colour to become blue.

When a section has been overstained with hæmatoxylin, the excess of colour may be removed by soaking it for a few minutes in a ½ per cent. solution of glacial acetic acid in distilled water, then wash again in tap water.

(4) Dehydrate in methylated spirit.

(5) Clear in clove oil, and mount in Canada balsam.

Double Staining with Hæmatoxylin and Eosin.—Stain the section in hæmatoxylin, as directed above, then place it in an alcoholic solution of eosin—about 1 grain of eosin to an ounce of methylated spirit is strong enough—and let it soak for about five minutes; wash well in methylated spirit, clear in clove-oil, and mount in Canada balsam.

Aniline Blue-Black.—Dissolve 30 grains of nigrosine in 3½ ounces of distilled water, then add 1 ounce of rectified alcohol and filter. This stain is only used for sections of brain and spinal cord. Immerse the sections for from thirty to sixty minutes, wash in water, dehydrate in methylated spirit, clear in clove-oil, and mount in Canada balsam.

Aniline Blue.—Make a 1 per cent. solution of soluble aniline blue in distilled water and filter. Stain the section for five to ten minutes, wash in water, and place in methylated spirit, in which it must soak until the excess of colour is removed. Clear in clove-oil and mount in Canada balsam.

This stain is useful for cardiac glands of the stomach, brain and spinal cord.

Golgi's Nitrate of Silver Methods.—These are chiefly employed for investigating the relations of cells and fibres in the central nervous system. Two methods are mostly used, as follows:

(*a*) Very small pieces of the tissue, which have been hardened for some weeks in bichromate solution or Müller's fluid, are placed for half an hour in the dark in 0·75 per cent. nitrate of silver solution, and are then transferred for

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twenty-four hours or more to a fresh quantity of the same solution (to which a drop or two of formic acid may be added). They may then be hardened with 50 per cent. alcohol, and sections, which need not be thin, are cut either from celloidin with a microtome or with the free hand. The sections are mounted in Canada balsam, which is allowed to dry on the slide. They must not be covered with a cover-glass, but the balsam must remain exposed to the air.

(*b*) Instead of being slowly hardened in bichromate, the tissue is placed at once in very small pieces in a mixture of bichromate and osmic (3 parts of Müller's fluid to one of osmic acid). In this it remains from two to five days, after which the pieces are treated with silver nitrate, as in the other case. This method is not only more rapid than the other, but is more sure in its results.

Mounting in Canada Balsam.—Take 3 ounces of dried Canada balsam and dissolve in 3 fluid ounces of pure benzol, filter, and keep in an outside stoppered bottle. Clear the section in clove-oil, and place it in turpentine. Clean a cover-glass and a slide, place a few drops of balsam on the centre of the latter, take the section from the turpentine on a lifter, allow the excess of turpentine to drain away, and with a needle-point pull the section off the lifter into the balsam on the slide. Now take up the cover-glass with a pair of forceps, and bring its edge in contact with the balsam on the slide; ease it down carefully, so that no air-bubbles are enclosed, and with the points of the forceps press on the surface of the cover until the section lies quite flat, and the excess of balsam is squeezed out. The slide must now be put aside for a day or two to allow the balsam to harden; the exuded medium may then be washed away with some benzol and a soft camel's hair brush, after which dry the slide carefully with a cloth and apply a ring of cement. The above method answers well for mounting sections quickly, but when time will admit the following is a much better way. Clear the section and place it in turpen-

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tine; clean a cover-glass, and moisten the surface of a slide with your breath; apply the cover-glass to the slide, and make sure that it adheres. Place a few drops of balsam on the cover, into which put the section. Now put the slide away in a box, or in some place out of reach of dust, for twelve hours, so that the benzol may evaporate from the balsam. Clean a slide, warm it gently over the flame of a spirit-lamp; apply a drop of balsam to the surface of the hardened balsam on the cover-glass; take the cover up in a pair of forceps, and bring the drop of fresh balsam in contact with the centre of the warmed slide. Ease the cover down carefully, so that no air-bubbles may be enclosed, press on the surface of the cover-glass until the section lies quite flat; set the slide aside to cool. The exuded balsam may then be washed away with methylated spirit and a soft rag, and a ring of cement applied.

Staining in Bulk.—Place small pieces of the tissue in Grenacher's alcoholic carmine for from one to three days, then transfer to a ½ per cent. solution of hydrochloric acid in methylated spirit for from one to twelve hours, according to the size of the tissue. Wash well in spirit, and soak for a day in 90 per cent. spirit.

The specimen may then be infiltrated and embedded in paraffin, celloidin or gelatine, but be careful to follow the instructions previously given with each method.

Flemming's Method for Staining Karyokinetic Nuclei.—Fix the tissue in the following Flemming's solution:

Osmic acid, 1 per cent. solution ...	80 c.c.
Chromic acid, 10 per cent. solution ...	15 c.c.
Glacial acetic acid	10 c.c.
Distilled water	95 c.c.

The fixing process is usually complete in twelve hours; then wash the tissue thoroughly in water and harden in alcohol of gradually increasing strength. Now place small shreds or thin sections in a saturated alcoholic solution of

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saffranin mixed with an equal quantity of aniline water for two days. The tissue is then to be washed in distilled water. It is then soaked in absolute alcohol until the colour is removed from everything except the nuclei. It is then again rinsed in water and placed in a saturated watery solution of gentian violet for two hours, washed again in distilled water, decolourized in alcohol until only the nuclei are left stained; then transfer to bergamot-oil, and mount in xylol balsam.

Weigert-Pal Method for the central nervous system, by which all medullated fibres are stained darkly, while the grey substance and any sclerosed tracts of white matter are left uncoloured. Pieces of brain or spinal cord which have been hardened in Müller's fluid are to be placed direct in gum-water and syrup and soaked for a few hours; then make sections with a freezing microtome, and place them in water, and from this transfer to Marchi's fluid, as follows:

Müller's fluid	...	...	...	2 parts,
Osmic acid, 1 per cent.		...	...	1 part,

and soak for a few hours. They are then washed in water and transferred to the following stain: Dissolve 1 gramme of hæmatoxylin in a little alcohol, and add to it 100 c.c. of a 2 per cent. solution of glacial acetic acid, in which leave the section for twelve hours; it will then be quite black. Wash again in water, and place in a $\frac{1}{4}$ per cent. solution of potassic permanganate for 5 minutes; rinse with water and transfer to Pal's solution (sulphate of soda 1 gramme, oxalic acid 1 gramme, distilled water 200 c.c.), and bleach for a few minutes. When sufficiently bleached they are passed through water into alcohol, cleared in bergamot-oil, and mounted in Canada balsam.

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Ehrlich's Triple Stain for Blood Corpuscles.

Saturated watery solution,	orange 'G' ...	135 parts.
,, ,, ,,	methyl green	110 ,,
,, ,, ,,	acid fuchsin	100 ,,

To the above add

Glycerine	100 parts
Absolute alcohol	200 ,,
Distilled water	300 ,,

This solution should stand for several weeks to allow for sedimentation, and it improves with age. When used the supernatant liquid should be drawn off with a pipette to avoid the sediment.

The cover-glasses are to be well cleaned with alcohol, and the surface of one is touched with a drop of fresh blood, and another cover-glass pressed on its surface until the blood is evenly distributed. The covers are then separated and allowed to dry. When dry they must be still further hardened over a spirit lamp, or on a hot stage made of sheet copper, and kept at 212° F. for from fifteen minutes to two hours; after which place in stain for from one to four minutes, wash in water, dry, and mount in Canada balsam, benzole, or xylol.

The eosinophile granules in the corpuscles will be a reddish hue, the neutrophile granules purple, and the nuclei bluish green or blue.

Toison's Solution for staining White Blood Corpuscles.

Methyl violet	½ grain.
Neutral glycerine	1 ounce.
Distilled water	2½ ounces.

Mix thoroughly and add

Chloride of sodium	15 grains.
Sulphate of sodium	2 drams.
Distilled of water	5½ ounces.

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Filter and keep in a stoppered bottle. Spread blood on cover-glass, dry, and immerse in stain for eleven minutes. Wash in water, dry, and mount in Canada balsam.

Fixing and Staining Sections on the Slide.

Mayer's Albumen Method. — White of egg 50 c.c., glycerine 50 c.c., salicylate of soda 1 gramme; shake well together, and filter into a stoppered bottle. A thin layer of the cement is spread on a slide with a brush, and the section laid on it. Now warm gently on a water-bath. As the paraffin melts it is carried away from the section by the albumen. The section may now be washed with turpentine, benzole, and alcohol, and be treated with aqueous or other stains, without fear of it moving.

Shellac Method.—Make a solution of shellac in absolute alcohol—it should be about the thickness of oil—filter, and keep in a stoppered bottle. Warm some slides, and spread over them a layer of the cement with a brush, and put away to dry. When dry apply a very thin layer of creasote; this will form a sticky surface, on which the section must be carefully laid. Now heat the slide on a water-bath for about fifteen minutes at the melting-point of the paraffin; this will allow the section to come down on the shellac film, and at the same time evaporate the creasote. Allow the slide to cool, and wash away the paraffin with turpentine or benzole. If the section has been stained in bulk, a drop or two of Canada balsam is added, and a cover-glass applied.

To Stain a Section on the Slide.—Fix section on slide as directed above. Wash away the paraffin with rectified mineral naphtha, follow this quickly with a few drops of methylated spirit, and then with some distilled water. Now apply the stain, and place the slide under a bell-glass to prevent evaporation; or the slide may be plunged into a vessel containing the staining solution. When sufficiently stained, wash with distilled water, dehydrate with methy-

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lated spirit, drain away the spirit, and apply a drop of clove-oil to clear the specimen. When clear, drain away as much of the oil as possible, add a drop of Canada balsam, and apply the cover-glass.

LESSON IV.

STAINING BLOOD AND EPITHELIUM, TEASING-OUT TISSUES, AND MOUNTING IN AQUEOUS MEDIA.

STAINING WITH PICROCARMINE, GOLD CHLORIDE, SILVER NITRATE, AND OSMIC ACID.

Double-staining Nucleated Blood Corpuscles.

Stain A.—Dissolve 5 grains of eosin in ½ ounce of distilled water and add ½ ounce of rectified alcohol.

Stain B.—Dissolve 5 grains of methyl green in an ounce of distilled water.

Place a drop of frog's blood on a slide, and with the edge of another slide spread it evenly over the centre of the slip; now put it away out of reach of dust to dry. When quite dry, flood the slide with stain A for three minutes. Then wash with water, and flood the slide with stain B for five minutes. Wash again with water, and allow the slide to dry. Apply a drop or two of Canada balsam and a cover-glass.

Blood of Mammals, Non-nucleated Corpuscles.

Spread a drop of blood on a slide and let it dry for twelve hours, then stain in a strong alcoholic solution of eosin for about five minutes, drain away the eosin, rinse the slide in methylated spirit, let it dry, apply a drop of Canada balsam and the cover-glass.

Both of the above processes should be carried out during

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dry weather, as any moisture in the air retards the drying of the corpuscles, and then they are liable to change their form.

Epithelium.—Kill a frog, cut off its head, and remove the lower jaw. Open the abdomen and take out the stomach, and slit it open. Place the head, lower jaw and stomach in a 2 per cent. solution of bichromate of potash for forty-eight hours. Then wash gently in water until no colour comes away from the specimens. Now place all three portions in picrocarmine for twenty-four hours. Remove the tissues from the carmine, and allow the stain to drain away from them. Take the lower jaw and scrape the tongue for squamous epithelium, and place the deposit obtained in a few drops of glycerine on a slide. Take the stomach, remove some columnar epithelium from its internal surface, and place it in some glycerine on another slide. Then take the head for ciliated epithelium, which will be found at the hinder part of the roof of the mouth ; put some scrapings from this in glycerine on a slide as before. Clean a slide and place a drop or two of Farrant's medium on its centre ; take up a little of the epithelium on the point of a needle, and put it into the medium. Now apply a cover-glass, and with the needle-point press it down until the epithelial cells are separated and spread evenly between the cover and the slide. Set the slide aside for a day or two, so that the medium may set. Then wash away the excess of medium with some water and a camel's hair brush, dry the slide with a soft rag, put it in a turn-table, and run on a ring of cement.

Portions of the tongue, trachea and intestine of a rabbit or cat may be treated in the same way.

Endothelium.—Take a piece of the omentum of any small animal, and rinse gently in distilled water to remove soluble matter. Place it in a $\frac{1}{4}$ per cent. solution of silver nitrate for ten minutes, or until it becomes a milky white. Wash well in ordinary water, and expose in a saucer of water to diffused sunlight, until it assumes a brownish colour. Cut out a small piece and mount it in Farrant's

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medium or glycerine jelly. In this specimen only the interstitial cement substance will be seen. To compare with it, cut out a similar piece, wash it in distilled water, and stain it with hæmatoxylin for ten minutes; wash away all excess of stain with distilled water, and mount in Farrant's medium or glycerine jelly. In this specimen the nuclei will be seen stained blue. Specimens of mesentery showing endothelium may also be mounted in Canada balsam. When this is desired, stain the tissue as directed above, dehydrate in methylated spirit, clear in clove-oil, and mount in Canada balsam.

Teasing-out Tissues.—Take a very small piece of the tissue, place it on a slide in a few drops of distilled water, and with a couple of needles mounted in holders carefully separate the fibres from each other. When the parts are sufficiently isolated, drain away the water, add a few drops of the mounting fluid, and apply the cover-glass. When teasing it is very important that a proper background should be used, so that the object may be easily seen. For a coloured specimen, a piece of white paper should be used, and a transparent white tissue will be seen better on a dark ground, such as a piece of black paper or American cloth; the slide should be examined from time to time under the low power of the microscope to ascertain when the tissue is teased out enough.

White Fibrous Tissue.—Harden some tendons from a rat's tail in methylated spirit for a week. Then soak a small piece in water to remove all trace of spirit, place it on a slide in a few drops of water, and tease it up until the fibres are separated from each other. Drain away the water, add some Farrant's medium or glycerine jelly, and apply a cover-glass.

Yellow Elastic Tissue.—Place small pieces of the ligamentum nuchæ of an ox in chromic acid and spirit for ten days. Then proceed as above.

Striped or Voluntary Muscle.—Harden small pieces of muscle of a pig in a 2 per cent. solution of bichromate of

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potash for three weeks, then transfer to methylated spirit, in which it may remain until required. Soak a piece in water to remove the spirit, place a very small fragment on a slide in a few drops of water, and with a couple of needles tease or tear the tissue up so as to separate the fibres. Drain away the excess of water, apply a drop or two of Farrant's medium and a cover-glass.

Non-striped Muscle.—Harden a piece of the intestine of a rabbit in chromic acid and spirit for ten days. Wash in water, strip off a thin layer of the muscular coats, and stain it in hæmatoxylin. Wash in distilled water, and then soak in ordinary tap-water until the colour becomes blue. Clean a slide, pass a small fragment of the muscle on it in a few drops of water, and with needles separate the fibres. Drain off the excess of water, apply a few drops of Farrant's medium and a cover-glass.

Nerve Fibres.—Dissect out the sciatic nerve of a frog, and stretch it on a small piece of wood as follows: Take a match, make a slit in each end of it, into which put the ends of the nerve; now place it in a 1 per cent. solution of osmic acid for an hour or two. Wash in water, tease up a small fragment on a slide, and apply a few drops of Farrant's medium and a cover-glass.

When staining with gold chloride, solutions from ½ per cent. to 5 per cent. in distilled water are employed. It is used for staining nerves and nerve-endings; it also brings out the cells of the cornea, fibrous connective tissues, and cartilage.

The tissue must be taken from the animal immediately after death, and be placed in the solution of gold for from half an hour to an hour; it is then removed to distilled water for twelve hours, and afterwards exposed to the action of diffuse sunlight in a saturated solution of tartaric acid or formic acid until it assumes a purple colour.

The future treatment will depend on the nature of the specimen.

If muscle has been stained for nerve-endings, place a

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small piece on a slide, tease it up, and examine with a low power until you find a nerve fibre terminating in an end-plate on a muscle-fibre, separate it from the surrounding fibres as much as possible, add some Farrant's medium or glycerine jelly, and apply a cover-glass.

If cornea or cartilage, make vertical and horizontal sections with a freezing microtome, and mount in Farrant's medium or glycerine jelly. Sections of gold-stained tissues may also be mounted in Canada balsam; when this is desired, dehydrate in strong spirit, clear in clove-oil, and mount in Canada balsam.

There are many ways of staining with gold, but the above is the most simple, and it gives very good results. For the other methods the student may refer to the larger works on practical histology.

Staining with Picrocarmine.—Rub up 1 gramme of carmine with 10 c.c. of water, and 3 c.c. of strong liquid ammonia; add this to 200 c.c. of a saturated solution of picric acid in distilled water. Leave the mixture exposed to the air until it evaporates to one-third of its bulk; filter, and keep in a stoppered bottle. Place some of the picrocarmine in a watch-glass, and immerse the section for from half an hour to an hour. Remove from the stain with a lifter, and place the section on a slide; drain away as much of the excess of stain as possible, and, if necessary, soak up what remains with a piece of filter-paper. Then add a few drops of Farrant's medium, and apply the cover-glass.

Picrocarmine stained tissues should never be washed; if they are, all the yellow colour will be removed, and the specimen will come out stained with carmine only. They improve by keeping, and the staining process goes on for several days after they are mounted; that is to say, some parts give up the stain, and others absorb it. Picrocarmine may be purchased in crystals, with which a 2 per cent. solution in distilled water should be made.

If it is desirable to mount a picrocarmine stained section

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in Canada balsam, proceed to stain as above; then make a saturated solution of picric acid in methylated spirit, filter, and dehydrate the section in it; then give it a final rinse in methylated spirit, clear in clove-oil and mount in Canada balsam.

Farrant's Medium.—Take of glycerine and a saturated aqueous solution of arsenious acid equal parts, and mix them well together; then add as much powdered gum arabic as the mixture will take up, and let it stand for six weeks. Filter, and keep in an outside stoppered bottle.

The above is difficult to make; it is better to obtain it ready for use.

Glycerine Jelly.—Dissolve 1 ounce of French gelatine in 6 ounces of distilled water; then melt in a water-bath, and add 4 ounces of glycerine and a few drops of creasote or carbolic acid. Filter through paper while warm, and keep in a stoppered bottle. The above may be used instead of Farrant's medium. The jelly must, of course, be warmed before use. All tissues or sections must be well soaked in water before they are mounted in Farrant's medium or glycerine jelly, so that all trace of alcohol is removed.

Tissues containing much air should be soaked in water that has been boiled for about ten minutes and allowed to cool.

LESSON V.

STAINING AND MOUNTING MICRO-ORGANISMS.

The investigation of bacteria may be carried out under various conditions.

(1) In fluids, such as milk, water, blood, pus, etc. (2) On solid media, bread, meat, potatoes, meat jelly, etc., or in the tissues and organs of animals. In the former case a drop of fluid is placed on the centre of a cover-

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glass, and another cover-glass is placed on it; the two glasses are then to be rubbed together to spread the organisms evenly over their surfaces; they are then separated and allowed to dry. When bacteria are growing on solid material, scrape off a small portion, put on a cover-glass, and treat as above; separate the covers, and allow to dry. When the cover is quite dry, take it up with a pair of forceps, organisms uppermost, and pass two or three times through the flame of a spirit-lamp; this will fix the albumen and fasten the bacteria to the glass.

To Stain Bacteria on Cover-glasses.—They should be floated with the organisms downwards on a saturated watery solution of any of the following aniline dyes: Methyl blue, methyl violet, gentian violet, fuchsin, vesuvin, or Bismarck brown. From ten to fifteen minutes is enough for the first four stains; vesuvin and Bismarck brown require about an hour. When the staining is complete wash the cover in distilled water. If the colour is too deep wash it in a ½ per cent. solution of acetic acid, and then again in water; put away to dry. When quite dry add a drop of Canada balsam, and mount on a slide in the usual way.

When bacteria are present in the organs of animals the tissues should be hardened in methylated spirit for about a week, and very thin sections with a freezing microtome cut from them. The sections may be stained in any of the above dyes; then wash in water, dehydrate in absolute alcohol, clear in oil of cedar or bergamot, and mount in balsam.

Staining Bacillus Tuberculosis.

Ehrlich's Method for Double-staining.—To 100 parts of a saturated watery solution of aniline oil add 11 parts of a saturated alcoholic solution of fuchsin, and filter. Place the covers or sections in the stain in a watch-glass, and warm slowly over a spirit-lamp until vapour rises. Wash in water, and then immerse for about a minute in dilute

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nitric acid, 1 part of acid to 2 parts water. Wash again in water, and stain again in a solution of methyl blue—100 parts of distilled water to 20 parts of a saturated solution of methylated blue—in alcohol for about twenty minutes. Wash in water, and in the case of sections dehydrate in absolute alcohol, clear in oil of cedar or bergamot, and mount in balsam. The cover-glass preparations must be dried; then add a drop or two of balsam, and mount as above.

Ziehl Neelsen's Method.—Fuchsin 1 part, 5 per cent. watery solution of carbolic acid 100 parts, absolute alcohol 10 parts. Remove the section from the alcohol, and immerse in the above stain for fifteen minutes.

Decolourize in a 5 per cent. watery solution of sulphuric acid, wash well in water to remove acid, and counter-stain in the following for five minutes: Saturated alcoholic solution of methyl blue 1 c.c., distilled water 5 c.c. Wash in water, dehydrate in absolute alcohol, clear in cedar-oil, and mount in Canada balsam.

Gibbe's Double Stain.—Rose aniline hydrochloride, 2 grammes; methyl blue, 1 gramme; rub well together in a mortar. Then dissolve aniline oil 3 c.c. in 15 c.c. of rectified spirit, and add the crystals to the mixture; shake well, and when all are dissolved, add 15 c.c. of distilled water. Place the cover-glass preparation or sections in the stain in a watch-glass, and warm gently over a spirit-lamp; then let them soak for four or five minutes. Wash in methylated spirit until no colour will come away, clear in oil of cedar, and mount in Canada balsam.

Cover-glass preparations will not require clearing; they are allowed to dry, then add a drop of balsam and mount on a slide.

Anthrax Bacillus.

Löffler's Alkaline Blue Method.—To 100 parts of a solution of caustic potash (1 in 10,000) in distilled water, add 30 parts of a saturated alcoholic solution of methylene

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blue. Immerse the sections for an hour. Wash in distilled water, and then in a ½ per cent. solution of acetic acid in distilled water. Wash away the acid with water, dehydrate in alcohol, clear in oil of cedar or bergamot, and mount in Canada balsam.

Anthrax bacilli may also be stained by Gram's method.

Gram's Method. — *Solution A.* — Saturated alcoholic solution of gentian violet, 11 parts; saturated watery solution of aniline, 100 parts. Mix well together, and filter.

Solution B.—Iodine, 1 part; iodide of potassium, 3 parts; distilled water, 300 parts.

Solution C.—Saturated aqueous solution of vesuvin.

Take the section from alcohol, and place in Solution A for one to three minutes. Wash in alcohol, and transfer to Solution B for three minutes. Wash in alcohol, and place in Solution C for five minutes. Wash in distilled water, dehydrate, clear in oil of cedar or bergamot, and mount in Canada balsam.

Leprosy Bacillus.—Stain in the following for three minutes:

Fuchsin	1 gramme,
Rectified spirit	20 c.c.,
Distilled water	80 c.c.,

then place for thirty seconds in a solution consisting of 90 per cent., alcohol 10 parts, and nitric acid 1 part. Wash in water, dehydrate in absolute alcohol, clear in cedar-oil, and mount in Canada balsam.

Diphtheria Bacillus.—Employ Löffler's alkaline blue and proceed as for anthrax.

Glanders Bacillus.

Kuhne's Method. — Methylene blue, 1 gramme; absolute alcohol, 10 c.c. When all the blue has dissolved, add 100 c.c. of a 5 per cent. watery solution of carbolic acid.

The sections are transferred from alcohol to the above stain for half an hour. Wash in water, and place in a weak

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solution of acetic acid in distilled water until they are of a pale-blue colour; watch carefully, or too much colour may be removed; they are then rinsed in lithia water (1 in 70) 1 c.c., water 34 c.c., and transferred to water. The sections are now to be taken up one at a time on the point of a needle and dipped into absolute alcohol, in which some methylene blue has been dissolved. Dehydrate in methylene aniline oil, made as follows: Rub up about 10 grammes of methylene blue with 10 c.c. of aniline, and let the mixture settle. When dehydrated, rinse in aniline, and place for a few minutes in terebene to clear, then mount in Canada balsam.

Schutz's Method.—Stain the sections or cover-glass films in methylene blue, 1 gramme; rectified spirit, 20 c.c.; distilled water, 80 c.c., for several hours. Wash in a ½ per cent. solution of acetic acid, dehydrate in absolute alcohol, and clear in cedar-oil, and mount in Canada balsam.

Syphilis Bacillus.—Stain by Lusgarten's method as follows:

Aniline-oil	3 c.c.
Distilled water	100 c.c.
Saturated alcohol solution of gentian violet	11 c.c.
Alcohol	10 c.c.

Sections or cover-glasses are placed in the above for from twelve to twenty-four hours. They are then transferred to absolute alcohol for a few minutes; then place for ten seconds in a 1 per cent. solution of permanganate of potassium, and wash in 5 per cent. solution of sulphuric acid to decolourize the ground tissue. Wash in water, dehydrate in absolute alcohol, clear in cedar-oil, and mount in Canada balsam.

Bacillus of Enteric Fever—Gaffky's Method.—Sections or cover-glasses are placed for twenty-four hours in a strong solution freshly made by adding a saturated alcoholic solution of methylene blue to distilled water. They

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are then washed in distilled water, dehydrated in absolute alcohol, cleared in terebene, and mounted in Canada balsam.

Spirillum.—On cover-glasses these are easily stained by any aniline dye solutions. When in sections use the following stain for twenty-four hours :

Bismarck brown	...	...	1 gramme.
Rectified alcohol	...	...	20 c.c.
Distilled water	...	...	80 c.c.

Then wash in water, dehydrate in absolute alcohol, and mount in Canada balsam.

Actinomycosis.

Stain the sections in the following for ten minutes, warmed to about 45° C.: Magenta, 2 parts; aniline-oil, 3 parts; rectified spirit, 20 parts; distilled water, 20 parts. Wash in water. Place in a concentrated alcoholic solution of picric acid for five to ten minutes. Wash in water, dehydrate in alcohol, clear in clove-oil, and mount in Canada balsam.

Weigert's Method.—Glacial acetic acid, 5 c.c.; absolute alcohol, 20 c.c.; distilled water, 40 c.c.; add orseille until a dark-red fluid is obtained. Stain the sections in the above for an hour; rinse quickly in alcohol. Clear in cedar-oil and mount in Canada balsam.

Hæmatozoa of Laveran.—Touch a drop of blood with a perfectly clean cover-glass, apply another cover-glass, press them gently together, then slide them apart, and dry. Now stain in an alcoholic solution of methylene blue, wash in water, dry and mount in Canada balsam.

Filaria.—Specimens are obtained by pricking the finger of the patient and applying a drop or two of blood to a glass slide; spread evenly with the aid of a thin glass rod, and allow it to dry. Now apply a few drops of Ehrlich's hæmatoxylin, and stain for about five minutes. Wash in distilled water, then stain again in a solution of eosine in alcohol, rinse in water, let the slide stand up on end to

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drain and dry, and then apply a drop or two of Canada balsam on the cover-glass.

Vermes.—Sections of specimens such as Acaris, Tænia, etc., may be made in the following way: Harden the worm in alcohol for a week or ten days. Then cut up in pieces of about ¼ inch long, and soak in equal parts of ether and alcohol for twelve hours; they are then transferred to a thin solution of celloidin in equal parts of ether and alcohol, and must remain in this until perfectly infiltrated. Now remove from thin celloidin and place in a thicker solution, and soak again for twelve hours. Remove from celloidin on the point of a needle, and hold exposed to the air for a minute or two so that the celloidin may dry all round the exterior of the specimen; then push it off the needle into methylated spirit, in which it should remain for twelve hours to complete the hardening of celloidin in the interior. Cut transverse sections, and stain in borax carmine for five minutes. Wash in methylated spirit, and then place in acidulated spirit—1 part hydrochloric acid in 5 of methylated spirit—for about three minutes if over-stained, until the excess of stain is removed. Wash again well in methylated spirit to remove all trace of acid. Then transfer, for about one to two minutes, to absolute alcohol, clear in oil of origanum, and mount in Canada balsam.

Great care must be taken not to leave the section in absolute alcohol for more than two minutes, or the celloidin will be dissolved and the section will fall to pieces.

Heads and segments of tape-worms, flukes, etc., may all be mounted whole. Harden in methylated spirit for a few days, then stain in borax carmine for from one hour to twenty-four hours according to the size of the specimen. Wash in methylated spirit, and soak in acidulated spirit until the excess of stain is removed. Then place in water for a few minutes to soften the tissue a little. Place the specimen on a glass slide, put another slide on it, and press down carefully until quite flat. Now bind the two slides

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together with twine and place in a jar of methylated spirit, and soak for at least twenty-four hours. Then remove the twine, separate the slides carefully, and place the specimen in absolute alcohol for ten minutes to dehydrate. Clear in clove-oil for one hour, and mount in Canada balsam.

Anchylostoma.—Harden in methylated spirit for ten days; then stain in borax carmine, wash in methylated spirit, and place in acidulated alcohol to remove excess of colour. Transfer to water, and soak until all trace of spirit is removed; then mount in glycerine jelly.

Trichina Spiralis. — Harden muscle with trichina encysted in methylated spirit. Then embed in celloidin, make longitudinal sections, stain in borax carmine, pass through acidulated spirit; then wash in water, and mount in glycerine jelly.

These worms may also be isolated from the muscle. Tear out a piece of muscle on a glass slide with the aid of a dissecting microscope or pocket lens, separate the capsule containing the trichina from the muscle with a needle, and place it in dilute hydrochloric acid until the capsule is dissolved and the worm is set free; then pick it up with a fine sable brush, wash in water, and mount in glycerine jelly.

LESSON VI.

INJECTION OF BLOODVESSELS.

Carmine and Gelatine Injecting Mass.—

Pure carmine	60 grains.
Liq. ammonia fort.	2 drams.
Glacial acetic acid	86 minims.
Gelatine solution (1 ounce in 6 ounces of water)	2 ounces.
Water	2 ounces.

Dissolve the carmine in the ammonia and water in a test-tube, and mix it with one-half of the warm gelatine. Add

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the acid to the remaining half of gelatine, and drop it little by little into the carmine mixture, stirring well all the time with a stick or glass rod. Filter through flannel, and add a few drops of carbolic acid to make the mass keep. The principle to be remembered in making this mass is this: the carmine, if alkaline, would diffuse through the vessels and stain the tissues around them; if acid, the carmine would be deposited in fine granules, which would block up the capillaries; hence the necessity for a *neutral* fluid. The best guides are the colour and smell of the fluid. It should be a bright red, and all trace of smell of ammonia must be removed. The gelatine solution is made by putting 1 ounce of gelatine into 6 ounces of water; it must then be left until the gelatine becomes quite soft; then dissolve over a water-bath.

Prussian or Berlin Blue and Gelatine Mass.—Take 1½ ounces of gelatine, place it in a vessel and cover it with water; allow it to stand until all the water is absorbed and the gelatine is quite soft. Then dissolve in a hot-water bath. Dissolve 1 dram of Prussian or Berlin blue and 1 dram of oxalic acid in 6 ounces of water, and gradually mix it with the gelatine solution, stirring well all the time; then filter through flannel.

Watery Solution of Berlin Blue.—Dissolve 2½ drams of the blue in 18 ounces of distilled water and filter. This fluid is useful for injecting lymphatics.

Injecting Apparatus Required.—An injecting syringe fitted with a stop-cock, and several cannulæ of various sizes.

Directions for Injecting.—The animal to be injected should be killed by chloroform, so that the vessels may be dilated, and injected while warm; if possible it should be placed in a bath of water at a temperature of 40° C. Expose the artery of the parts to be injected, clear a small portion of it from the surrounding tissues, and place a ligature of thin twine or silk round it. With sharp scissors make an oblique slit in the wall of the vessel, insert the

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cannula, and tie the ligature firmly over the artery behind the point of the cannula, into which put the stop-cock. Fill the syringe with injection fluid, which must not be too warm, and take care not to draw up any air-bubbles; now insert the nozzle of the syringe into the stop-cock and force in a little fluid; remove the syringe, so that the air may escape, insert the syringe again, and repeat the process until no air-bubbles come out of the stop-cock. You may then proceed slowly with the injection. Half an hour is not too long to take over the injection of an animal of the size of a cat. The completeness of an injection may be judged by looking at the vascular parts, such as the tongue, eyelids, and lips. When the injection is complete shut the stop-cock, remove the syringe and cannula, and tie the ligature round the artery. Now place the animal in cold water for an hour to set the injection-fluid. When quite cold, dissect out the organs, cut them up into small pieces, and place them in methylated spirit to harden, and change the spirit every twenty-four hours for the first three days. The hardening will be complete in ten days.

Injection of Lymphatics (*Puncture method*).—A small subcutaneous syringe is filled with a watery solution of Berlin or Prussian blue, and the nozzle is thrust into the pad of a cat's foot. The injection is to be forced into the tissues. Then rub the limb from below upwards. This will cause the injection-fluid to flow along the lymphatics, and find its way into the glands of the groin.

To Inject Lymph-sinuses of Glands.—Force the nozzle of a subcutaneous syringe into the hilum of a lymphatic gland of an ox, and inject a watery solution of Prussian or Berlin blue until the blue appears on the surface of the gland. Then place it in methylated spirit to harden.

When *blue* injection-fluid is used, add a few drops of acetic acid to the spirit while hardening the tissues.

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LESSON VII.

CUTTING, STAINING, AND MOUNTING VEGETABLE SECTIONS.

Stems, leaves, roots, etc., should be hardened in methylated spirit for a week or ten days, and the spirit changed every twenty-four hours for the first three days. The stems must not be too old. One, two, and three years' growth will show all that is required.

Wheat, barley, maize, peas, etc., are usually obtained dry. They must be placed in water for a few hours or until they resume their natural shape. Then lay a piece of blotting-paper on a plate, moisten it with water, and spread a layer of the grains on its surface; now place another piece of wet blotting-paper over all, and put in a warm place for from twelve to twenty-four hours, so that the embryo may begin to germinate. Then remove from the plate, and place the grains in a bottle of methylated spirit, which must be changed every day until all trace of water is removed. The specimens may then be sectionized or they may remain in spirit until required.

Ovaries.—Gather some before the flower opens, and others after it has been open for a day. You will then have the ovules in both stages. Place them in methylated spirit and change every twenty-four hours for the first three days.

Anthers.—Treat in exactly the same way as ovaries, but anthers must be infiltrated with celloidin before the sections can be cut. Remove the ends, place in equal parts of alcohol and ether, and soak for twelve hours; then place in celloidin, and, after soaking for from twelve to twenty-four hours proceed as directed in Lesson No. II. on Section Cutting.

Some specimens after being in spirit are too hard to cut easily. They may be softened by soaking in warm water. Leaves are often particularly troublesome in this respect;

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they bend and become fixed by the action of the spirit, and will not then stand the slight pressure required to hold them firmly between the carrot without cracking. When this happens, soak the leaf in warm water until it is quite pliable; it can then be embedded in carrot without any risk of being broken. Stems and petioles of many palms are naturally too hard, and they may contain a large amount of silica. They must be soaked in water for a while; then transfer to liq. potassæ for from one to twelve hours. Wash again well in water to remove all trace of potash, then reharden in methylated spirit. The shells of many stone fruits may be softened and cut by this method.

Section-cutting, by hand and with a microtome, should be done in the same manner as described in Lesson II.

Bleaching.—Vegetable sections generally require bleaching before they can be properly stained. Chlorinated soda is used for this purpose. Take of dry chloride of lime 2 ounces, of washing soda 4 ounces, and distilled water 2 pints. Mix the lime in 1 pint of the water and dissolve the soda in the other. Mix the two solutions together, shake well, and let the mixture stand for twenty-four hours. Pour off the clear fluid, filter, and keep in a stoppered bottle in a dark place, or cover the bottle with paper. Soak the sections in distilled water. Pour off the water and add a quantity of bleaching fluid. Allow this to act for from one to twelve hours. Wash well in water, which must be changed several times to remove all traces of soda. The sections may now be stained, or they may be preserved in spirit until required.

Staining Borax Carmine (suitable for ovaries, fruits, etc.).—Pure carmine 1 dram, liq. ammoniæ fort. 2 drams. Dissolve the carmine in the ammonia, and add 12 ounces of a saturated solution of borax in distilled water. Filter and keep in a stoppered bottle.

(1) Put some stain in a watch-glass, and immerse the section for three to five minutes.

(2) Wash well in methylated spirit.

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(3) Take of hydrochloric acid 1 part, and of methylated spirit 5 parts; mix well together, and soak the section until the colour changes to a bright scarlet, which takes about five minutes. The acidulated spirit may be kept ready for use at any time.

(4) Wash well in methylated spirit. Then place in some strong methylated spirit, and soak for at least ten minutes to dehydrate.

(5) Place the section on the surface of a small saucer of clove-oil, and let it soak until clear.

(6) Remove from the clove-oil and place in turpentine, and then mount in Canada balsam.

Full instructions for mounting in Canada balsam are given at end of lesson.

Hæmatoxylin. — Hæmatoxylin, 30 grains; absolute alcohol, 3½ ounces; distilled water, 3½ ounces; glycerine, 3 ounces; ammonia alum, 30 grains; glacial acetic acid 3 drams. Dissolve the hæmatoxylin in the alcohol and the alum in the water; then add the glycerine and acetic acid. Mix the two solutions together, and let the mixture stand for at least a month before use.

(1) Add about 30 drops of the above to an ounce of distilled water, and stain the section for fifteen to thirty minutes.

(2) Wash well in distilled water, and then in ordinary tap-water. This will fix the colour and make it deeper.

(3) Dehydrate in strong methylated spirit for at least ten minutes.

(4) Clear in clove-oil and mount in Canada balsam.

Double Staining—Dalton Smith's Method.—Stems, roots and leaves:

Green Stain.—Acid aniline green	...	2 grains.
Distilled water	...	3 ounces.
Glycerine ...	...	1 ounce.

Mix the water and glycerine together, and dissolve the green in the mixture.

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Carmine Stain A.—Borax	... ...	10 grains.
Distilled water	...	1 ounce.
Glycerine ...	...	½ ounce.
Alcohol rect.	...	½ ounce.

Dissolve the borax in the water, and add the glycerine and alcohol.

B.—Carmine	... ...	10 grains.
Liq. ammoniæ	...	20 min.
Distilled water	...	30 min.

Dissolve the carmine in the water and ammonia. Mix A and B together and filter.

(1) Place the section in green stain for five to ten minutes.

(2) Wash in water.

(3) Place in carmine from ten to fifteen minutes.

(4) Wash well in methylated spirit.

(5) Dehydrate and clear in clove-oil. Wash in turpentine and mount in Canada balsam.

Double Staining—M. J. Cole's Method.

Pure carmine	... ...	1 dram.
Liq. ammonia	... ...	2 drams.

Dissolve the carmine in the ammonia and add 12 ounces of a saturated solution of borax in distilled water. Filter through paper and keep in a stoppered bottle.

Bleach the sections, and after being well washed with repeated changes of water they are placed in the above stain for five to ten minutes. Then wash well in methylated spirit, and soak in acidulated alcohol—1 part hydrochloric acid to 5 of methylated spirit—until the excess of stain is removed; about two minutes is usually sufficient. Wash again in methylated spirit to remove all trace of acid. Dissolve 5 grams of acid aniline green in 6 ounces of methylated spirit, and filter if necessary. Soak the section in this green

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stain for at least half an hour; then just rinse in methylated spirit, clear in clove-oil, and mount in Canada balsam. The advantage of this method is that the section can remain in the green stain for any time. The writer keeps a stock of sections in it ready for mounting. Should a specimen be overstained green, the excess of colour can easily be removed by soaking in methylated spirit for a few minutes.

Staining with Eosin.—Make a 2 per cent. solution of eosin in alcohol, filter if necessary, and keep in a stoppered bottle. This stain is used for showing the structure of sieve-tubes and plates; it stains protoplasm deeply. Make transverse and longitudinal sections of the stem of a vegetable marrow, and immerse them in the above for ten minutes. Then wash out any excess of colour with methylated spirit, clear in clove-oil, and mount in Canada balsam.

Staining Hairs on Leaves.—Make a 2 per cent. aqueous solution of soluble aniline blue and filter. Now take, for example, a young leaf of *Deutzia scabia*, cut it into small pieces of about ¼ inch square, and bleach in chlorinated soda. Then wash well in water, and immerse in the above stain for twelve hours, wash well in water, and transfer to methylated spirit, in which they must be soaked until nearly all the colour is removed. Then soak in clove-oil for several hours, and when quite clear mount in Canada balsam.

Leaves of eucalyptus and other plants showing essential oil glands may be treated in the same way, but if the specimens have been preserved in spirit they must be soaked in water before the bleaching process.

Male and Female Conceptacles of Fucus and other Algæ.—Place the specimens in methylated spirit, which must be changed every twenty-four hours for the first three days, then let them soak for ten days or until required for cutting into sections.

Embed a conceptacle in carrot, place in microtome, and make transverse sections which must be as thin as possible.

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While cutting keep the knife well wetted with methylated spirit, and, as the sections are cut, put them into spirit; no water must come near them. When ready, stain the sections in a strong solution of acid aniline green in spirit for several hours. Then just rinse in absolute alcohol, clear in clove-oil, and mount in Canada balsam.

Transverse and longitudinal sections of the thallus of an alga may be treated in the same way, but they may be mounted without staining, as the tissues are coloured naturally brown and yellow.

Ovaries of Flowers.—Make transverse sections, which should be as thin as possible, and stain them either in borax carmine or hæmatoxylin, then clear in clove-oil, and mount in Canada balsam.

Anthers must be infiltrated with celloidin to keep the pollen in position. Then embed in carrot, place in microtome, and cut transverse sections. Stain in borax carmine, and after having passed through acidulated spirit, wash well in methylated spirit, and dehydrate for about one to two minutes in absolute alcohol; then clear in oil of origanum or bergamot, and mount in Canada balsam.

Flower Buds.—Infiltrate with celloidin as directed in Lesson No. II. on Section Cutting. Embed the specimen in carrot, and place in the microtome. Cut transverse sections, stain in borax carmine, and pass through acidulated spirit to remove excess of colour; if desired they may be soaked until the stain is removed from everything except the nuclei. Wash well in methylated spirit, and place in absolute alcohol for from one to two minutes; then clear in oil of origanum or bergamot, and mount in Canada balsam. Great care must be taken that the sections do not remain too long in absolute alcohol; if they should, the celloidin will dissolve, and the sections will fall to pieces.

Pollens.—Place some mature anthers in a large pill-box, and allow them to become perfectly dry. Shake the box well until all the pollen is set free; then remove the anther

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sacs with a pair of forceps, and place the pollen in a bottle of turpentine; soak for several days to remove all trace of air, then pour off the turpentine; take up a little of the pollen on the point of a penknife, and place it in a few drops of Canada balsam on a cover-glass; stir up with a needle to spread the grains evenly over the cover, and put away to dry. When the balsam has dried, add a few more drops of balsam, take up the cover with a pair of forceps, and mount it on a warm slide. This method of mounting must always be employed for pollens, because, if they are put up in any other way, the balsam only hardens at the edge of the cover, and remains in a more or less fluid state in the centre, with the result that, if the slide were placed on its edge, the specimens would run together in a heap at the lower side of the cover.

Pollens may also be mounted as opaque objects; see Lesson XIII. on Dry Mounts.

Pollens may also be stained various colours by aniline dyes. Place some fresh pollen in methylated spirit, and soak until air and most of the colour is removed. Then pour off the spirit and add a strong alcoholic solution of some aniline dye of the desired colour; any will do so long as it is soluble in alcohol. Soak in the dye for an hour or two, then pour off the stain, just rinse in spirit, pour this away, and add clove-oil, and when clear pour off the oil; take up a little pollen on the point of a knife, and mount in Canada balsam as directed for unstained specimens.

Specimens of pollens are sometimes stained many colours on the same slide. This is done in the following way: Take some pollen and divide it into equal quantities, each one of which is to be stained in a different dye. Then when they have been cleared by the clove-oil they are all mixed together and mounted in balsam.

Pharmacological Specimens.—Students of pharmacy may desire to make sections of the dried stems, roots and leaves with which they deal. Place the dry specimen in water, and soak until it resumes as nearly as possible its

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natural shape. Then place in methylated spirit, which must be changed every twenty-four hours for three days to remove all the water. Then make sections in accordance with instructions given for ordinary botanical specimens.

Powdered Drugs.—Place some of the powder in methylated spirit, and soak for an hour or two; then pour off the spirit, and add clove-oil; let it stand a little while, then drain off the oil, take up some of the powder on the point of a knife, place it in some Canada balsam on a cover-glass, mix it well up with the balsam, and then proceed to mount it as directed for pollens.

Mounting in Canada Balsam.—Take 3 ounces of dried Canada balsam and dissolve in 3 fluid ounces of benzole. Filter and keep in an outside stoppered bottle.

(1) Clean a cover-glass, moisten the surface of a slide with the breath, apply the cover-glass to it, and make sure that it adheres.

(2) Place a few drops of balsam on the cover-glass.

(3) Take the section out of the turpentine on a lifter, and put it into the balsam on the cover.

(4) Put away out of the reach of dust for twelve hours, to allow the benzine to evaporate from the balsam.

(5) Warm a slide over a spirit-lamp and apply a drop of balsam to that on the cover-glass; take it up with a pair of forceps, and bring the drop of fluid balsam in contact with the centre of the warmed slide. Ease the cover down carefully, so that no air-bubbles may be enclosed, and press it down with the point of the forceps until the section lies quite flat and the excess of balsam is squeezed out. Allow the slide to cool, and the excess of balsam may then be washed away with some methylated spirit and a soft rag.

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LESSON VIII.

THE PREPARATION OF VEGETABLE TISSUES FOR MOUNTING IN GLYCERINE JELLY, ACETATE OF COPPER SOLUTION, ETC.

Epidermis for Stomata.—Take a leaf, remove the edges with a pair of scissors, and then cut the remainder up into small pieces of about ¼ inch square. Place these in a test-tube, add nitric acid, and boil gently over a spirit-lamp for about a minute, then add a few grains of chlorate of potash, and bring to the boiling-point again. Pour away the acid and add water, which must be changed several times until all trace of acid is removed. The epidermis will then be found quite clean, and it may be stained and mounted at once, or be placed in spirit and kept until required.

Another Way.—Some epidermal tissues are very delicate and will not stand the acid treatment. When this is the case cut the leaf up as directed above, place the pieces in a jar of water, and put aside for a week or two. The action of water will rot the cellular tissue and set the epidermis free. Then wash well in water, and should any particles of débris adhere, they can be removed by brushing with a camel's hair brush.

The epidermis of some plants will not stand either of the above processes. When this is the case, the only plan is to strip off a small piece of the cuticle, lay it on a slide, inner side uppermost, and with a scalpel carefully scrape away cellular tissue that may be adhering. Then wash in water, and proceed with the staining.

To Stain the Epidermis.—Make a 1 per cent. solution of methyl aniline violet in distilled water and immerse the specimen for about five minutes. Then wash in a ½ per cent. solution of glacial acetic acid to remove excess of colour, wash away all trace of acid with water, and mount in glycerine jelly in the following way:

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Warm the jelly carefully in a water-bath until it is quite fluid. Warm a slide, take up a little jelly in a dipping-tube, and place it on the slide; now take up the epidermis with a lifter and put into the jelly on the slide, being very careful to avoid making any air-bubbles. Now take the cover-glass and apply it to the surface of the jelly, push down the cover with the point of a needle until it is quite flat, and then set aside to cool. The above process applies to all specimens that are to be mounted in jelly; but when tissues have been preserved in spirit they must be soaked well in water before being mounted.

Annular Vessels.—Get some stem of maize, cut it into pieces about half an inch long, and then cut again into thin longitudinal slices; place these in water until rotten. Now put some of the broken-up material on a slide and examine with a microscope; pick out the annular vessels on the point of a needle, place them in some clean water, and wash well. Stain in a weak watery solution of acid green, and after washing in water, mount in glycerine jelly.

Scalariform Vessels.—Treat pieces of the rhizome of Pteris aquilina in exactly the same way as stem of maize.

Spiral Vessels.—Treat pieces of the stem of rhubarb in the same manner as annular vessels.

Raphides may be isolated, or they can be mounted, *in situ*, in the tissues in which they occur. For the former, take some leaves of cactus, stem of rhubarb, and root of Turkey rhubarb, cut them up into thin slices longitudinally, and place them in a jar of water, covered up to keep out dust, and put away until the tissue has become perfectly disintegrated. This will take several weeks, and the process is more easily carried out by keeping the jar in a warm place. When all the material has broken up, stir well with a glass rod, and strain through a piece of coarse muslin into a shallow vessel, such as a soup-plate; stir up again, and then allow to settle for a minute, so that the raphides may fall to the bottom of the plate; now pour away as much of the dirty water as possible, add more clean water,

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and repeat the process until you have got rid of all the disintegrated vegetable fibre. Now pour the raphides into a bottle, and if they are quite clean, pour off the water and add methylated spirit, in which they may be preserved until required for mounting.

To mount isolated raphides, clean a cover-glass, fasten it to a slide with the aid of your breath, take up some of the raphides in a dipping-tube, place them on the cover-glass, and spread them evenly over its surface with a needle. Place the slide out of reach of dust until all the spirit has evaporated, and the raphides are quite dry; add a few drops of Canada balsam, and put the slide away again for twelve hours; then add a few drops more balsam, take up the cover with a pair of forceps, and mount it on a warmed slip. When the raphides are very large, they must be mounted in balsam that is rather thicker than is usually used.

Raphides in situ in Tissues.—Harden the stems, roots, or leaves, in methylated spirit, and make sections in the ordinary way; dehydrate, clear in clove-oil, and mount in Canada balsam.

Raphides in Scale-leaves of Bulbs, such as Onion, Garlic, Lily, Hyacinth.—Strip off a thin portion of the cuticle, place it in methylated spirit for a few hours, and when dehydrated clear in clove-oil and mount in Canada balsam.

Sometimes raphides are rendered too transparent when mounted in balsam. When this is the case they must be put up in glycerine jelly in the following way:

Isolated Specimens.—Pour off the methylated spirit, and add water; pour off the water, leaving the raphides at the bottom of the bottle. Clean a cover-glass and a slide. Place a few drops of warmed glycerine jelly on the centre of the slide; take up a few of the raphides on the point of a penknife, and place them in the glycerine jelly, but do not stir them up. Now apply the cover-glass, and press it down carefully with a needle, giving it at the same time a twisting

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motion, to spread the raphides evenly between the cover and slide. Put away for an hour or two, scrape off the excess of jelly with a penknife, wash in water, and then in methylated spirit, dry with a cloth, and apply a coat of black enamel. When raphides in the tissues are prepared in glycerine jelly, wash away all trace of spirit with water, and mount in glycerine jelly as above.

Starches (*Isolated Specimens*).—If the tissue is fresh, scrape the cut surface with a knife, and place the scrapings in a bottle of water; shake well and then strain through fine muslin into a shallow vessel; let the starch settle, pour off the water, and wash again with some clean water until the starch is quite clean; then place it in a bottle, and when it has settled to the bottom, pour off the water, and add methylated spirit.

Dried Specimens.—Place in water until the tissue swells up, then, if the material is large enough, it may be scraped and treated as above. If too small—small seeds, for instance—place them in a mortar in some water, and carefully break them up; strain through muslin, wash with water until quite clean, and preserve in methylated spirit.

Starches may be mounted in Canada balsam or glycerine jelly. If the former is chosen, spread a little starch evenly on a cover-glass, let it dry, apply some Canada balsam, and mount it in the ordinary way. For glycerine jelly pour off the spirit and add water, then allow the starch to settle to the bottom of the bottle; pour away the water. Place a few drops of glycerine jelly on a slide, take up some starch on a penknife, and place it in a little heap in the jelly; now apply a cover-glass, and press down with a gentle twisting movement until the starch is evenly spread. Let the jelly set, scrape away the excess, wash in water, then in spirit, dry, and apply a coat of cement.

It is desirable also to prepare specimens of starch *in situ* in the tissues. Take, for example, a potato, cut it into small pieces of about half an inch square, and harden them in methylated spirit. Then embed in carrot and cut the

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sections, which should not be too thin. Stain in a 1 per cent. solution of methyl aniline violet, wash in water, and mount in glycerine jelly.

In mounting starch in glycerine jelly, care should be taken that the jelly is not too hot; if it be, the form of the starch will be altered.

Yeast.—Get some fresh baker's yeast, place a little of it in a bottle of sugar and water, and stand in a warm place for twenty-four hours. Pour off the sugar-water, and add camphor-water. Make a cell on a slide with black shellac cement, and let it dry; then apply a second coat of cement, and let this stand for a few minutes. Now take up some of the yeast in a glass tube and place a few drops in the cell; clean a cover-glass, and bring its edge in contact with the cement on one side of the cell; ease it down carefully, so that no air-bubbles may be enclosed; now press on the surface of the cover with a needle until it adheres firmly to the cell all round, drain off the excess of fluid, dry the slide with a clean cloth, and apply a coat of cement.

Mycetozoa or Myxomycetes.—Most of these fungi can be mounted in glycerine jelly after soaking in equal parts of rectified spirit and glycerine to remove the air, but in those forms which possess lime granules in the capillitium —a character of importance in classification—the calcareous matter disappears when in glycerine in any form. When this is the case, place the specimen in absolute alcohol until all air is removed, then transfer to clove-oil, and mount in Canada balsam. Some specimens may, however, be rendered too transparent by the balsam; if so, mount them in a shallow cell in some neutral fluid such as camphor water.

In their ripe condition they may also be mounted dry as opaque objects.

Large fungi, such as agaricus, should be hardened in methylated spirit for a week. Then place the desired portion in water, and soak to remove spirit, transfer to gum and syrup, and when penetrated with the gum, freeze

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and make the sections with a Cathcart microtome, wash away all trace of gum with repeated changes of warm water, and mount unstained in glycerine jelly.

Preserving Fluid for Green Algæ.—Acetate of copper 15 grains; camphor water, 8 ounces; glacial acetic acid, 20 drops; glycerine, 8 ounces; corrosive sublimate, 1 grain. Mix well together, filter, and keep in a stoppered bottle. The above fluid preserves the colour of chlorophyll for a long time; it may also be used as a mounting fluid. For very delicate specimens leave out the glycerine.

The specimens should be well washed in water; then pour off the water, and add a quantity of the copper solution.

To Mount in the Above.—For example, take spirogyra as a filamentous alga. Make a cell with some black cement, and let it dry; then apply a second coat of cement, and allow this to nearly dry. Place some spirogyra in the cell, and with needles separate the filaments; add a few drops of copper solution, and apply a cover-glass as directed for yeast.

Protococcus.—This can be obtained by scraping the bark of trees. Place it in a bottle of water, and let it stand for a few hours; now add a little copper solution—this will kill the specimens, and they will sink to the bottom of the bottle; pour off the water, and add more copper solution. Now make a cell as for spirogyra; take up some of the protococcus in a dipping-tube, and place them in the cell; wait a minute for the forms to settle on the bottom of the cell, and then apply a cover-glass; drain off the excess of fluid, dry the slides with a cloth, and apply a coat of cement.

Volvox, glœocapsa, desmids, etc., may all be preserved and mounted as above.

Antheridia and Archegonia of Mosses.—Place some male and female heads of mosses in methylated spirit for a few days, then transfer to equal parts of absolute alcohol and ether, in which they must be soaked for several hours.

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Pour off the alcohol and ether, and add a thin solution of celloidin, and soak for two or three days; then remove the stopper of the bottle, and let the celloidin evaporate to about half its original bulk. Now remove a specimen from the celloidin, and hold it in a pair of forceps until the celloidin sets, then place it in methylated spirit and soak for an hour or two to complete the hardening. The embedded specimen may now be fastened to a cork with a little celloidin, and longitudinal sections made in a Cathcart microtome, or it can be placed between two pieces of carrot, and the sections made with any ordinary well microtome. The sections must then be dehydrated in methylated spirit, cleared in oil of bergamot, and mounted in Canada balsam; or, if desired, they may be soaked in water to remove spirit, and be mounted in glycerine jelly.

Fertile Branch of Chara.—Chara is usually very dirty; to clean it, wash well in repeated changes of water, then in very dilute acetic acid for a few minutes only; again wash in water, and preserve in camphor water.

Make a cell with shellac cement as directed above, place a fertile branch of chara in it, and examine under a dissecting microscope or lens; with needles clear away the leaves from the archegonia and antheridia, fill the cell with camphor-water, and apply a cover-glass.

When a deep cell is required for a specimen to be mounted in acetate of copper, never use one made of any metal. Vulcanite or glass cells must be used. To one side of a cell apply a coat of shellac cement and let it dry; now take a slide and warm it over a spirit-lamp; take up the cell in a pair of forceps, and bring the cemented side in contact with the centre of the warmed slide, and press it down until it adheres firmly; then add another coat of cement to the upper side of the cell, and let it nearly dry, put in the specimen, fill the cell with solution, and apply the cover-glass.

Prothallus of Fern.—Preserve in acetate of copper and mount in the same fluid in a shallow cell.

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Sporangia and Spores of Fern.—Place leaves of a fern with sporangia in methylated spirit for a few days to remove the air. Then soak in water for several hours. Warm a slide, and place a few drops of glycerine jelly on its surface, scrape off some sporangia, and place them in the jelly; now apply the cover-glass very carefully to avoid scattering the sporangia. The object is to keep them in a heap in the centre until the cover is flat; then press on the surface of the cover with the points of the forceps, and, if possible, give the cover a little twisting motion. This will spread the specimens; it will also rupture some of the sporangia and let out the spores.

Isolating Antheridia and Oogonia from Fucus.—Take some conceptacles that have been hardened in methylated spirit, and make thick sections by hand only with a sharp knife. Place these in a strong solution of acid aniline green in spirit, and let them stand for two or three hours. Now place in water for a few minutes, and they will at once swell up like a mass of mucus. Place this on a slide and put another slide on top of it, press down the upper slide—this will squeeze out the contents of the conceptacles in little round masses. Separate the glasses, pick up one of the little lumps of antheridia or oogonia, place it in a few drops of glycerine jelly on a slide, then apply the cover-glass, which must be pressed down to spread the specimens.

Digestive Glands in Pitcher Plant.—Harden some strips of a pitcher in methylated spirit for a week. Then place in water and soak for a few hours. Then lay the tissue with the glandular surface next to the glass, and with a scalpel scrape away the outer wall. Now bleach the glandular portion in chlorinated soda, then wash well with water, stain in aqueous solution of acid aniline green, wash again in water to remove excess of colour, soak for several hours in dilute glycerine, and mount in glycerine jelly.

Aleurone.—Take the endosperm of a castor-oil seed,

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embed in carrot, place in microtome, and cut sections as thin as possible with a knife wetted with a little olive-oil. As the sections are cut, put them on a slide, and place out of reach of dust until you are ready to mount them.

Make a shallow cell as directed with black enamel and let it dry, then proceed as directed for acetate of copper mounting, but use castor-oil instead of copper solution. When the cover has become fixed, wash away the exuded oil with a soft brush and some turpentine, and, when dry, apply a good finishing coat of black enamel. Water and spirit are apt to injure the aleurone grains, so they should be avoided.

Marine Algæ.—The best place for collecting specimens is a rocky shore, and the most suitable time is when the tide is at its lowest. As a rule, the inshore weeds near high-water mark are green, lower down there is usually a belt of olive forms sheltering red plants beneath them, and where rocks overhang small shallow pools red forms also occur at this level. At extreme low-water mark and beyond it are found brown tangles sheltering red plants again, while at the lowest depths the red weeds occur without shelter. The specimens will be found by searching the rocks and pools, some will be growing on pebbles and on shells, others will be attached to rocks, and varieties may be found stranded on the shore thrown there by waves, particularly after a storm, the tufts having been torn away and carried inshore from inaccessible regions.

For collecting, small tin boxes or an ordinary sponge bag will be found most suitable. A strong chisel mounted on a stout stick will also be required for removing specimens from rocks that are out of reach.

Many specimens may be preserved in sea water for a considerable time, but, as a rule, the sooner they are mounted the better.

Mounting Process.—Remove the specimen from sea water and wash well in fresh water. Place in a shallow

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white dish or saucer, select and cut off the portion that is to be mounted, and place it on a slide slightly warmed, drain away as much water as possible, and apply some glycerine jelly; then, if necessary, lay or spread out the leaves or filaments with a needle and apply the cover-glass, allow the slide to cool, remove the excess of jelly around the edge of the cover, wash the slide in water, dry, and add several coats of enamel or varnish.

Corallines, whose tissues are hard and opaque, may be cleaned by soaking for a short time in a weak solution of hydrochloric acid, then wash well in water, and mount in glycerine jelly.

LESSON IX.

CUTTING, GRINDING, AND MOUNTING SECTIONS OF HARD TISSUES. PREPARING METAL SPECIMENS.

Bone.—Take the femur of a dog or cat, remove as much of the muscle as possible, and macerate in water until quite clean, then allow it to dry.

(1) With a fine saw make transverse and longitudinal sections.

(2) Take a hone (water of Ayr stone), moisten it with water, and rub one side of the section upon it until it is quite flat and smooth.

(3) Wash in water, and set aside until quite dry.

(4) Take some dried Canada balsam, place a piece on a square glass, and warm gently over a lamp until the balsam melts; allow it to cool a little, and then press the smooth side of the section into it, and set aside until cold.

(5) With a fine file rub the section down as thinly as possible.

(6) Take the hone again and grind the section down until thin enough, using plenty of water.

(7) Place it, with the glass, in methylated spirit until the

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section comes away from the glass, then wash well in clean water.

(8) Make a cell with black shellac cement and allow it to dry; then apply another coat of cement, and after waiting a few minutes fill the cell with camphor water, and put the section into it; apply a cover-glass, and press it down until it adheres to the cement all round. Sections of teeth are made in the same way.

Rock Sections.—Small pieces or slices of rock are to be ground on a zinc plate with the aid of emery-powder and water until one side is quite flat and smooth. Then fasten the polished surface to a square of glass with some dried Canada balsam, as directed for bone, and allow it to cool. Grind the other side on the zinc plate with coarse emery and plenty of water. When moderately thin, take a piece of plate-glass and some fine flour-emery, and rub the section down as thinly as possible. When thin enough, wash well in water and dry; then warm over a spirit-lamp, and with a needle push the section off the glass into a saucer of benzole or turpentine, and allow it to soak until all the balsam is dissolved. Wash again in some clean benzole, and mount in Canada balsam in the usual way. Sections of echinus spines, shells, and stones of fruit are prepared in the same way as bones and teeth; but when the grinding is finished, the sections are to be passed through alcohol into clove-oil, then mount in Canada balsam in the usual way.

Sections of coal containing fossils, limestone, spines of echinus, and other friable specimens should be cut with a very fine saw, and then soaked in benzole for several hours. When the benzole has saturated the tissue, transfer to ordinary solution of Canada balsam in benzole, and soak again until the balsam has penetrated to the centre. Take a 3×1 inch slide, place the section on its centre, and add sufficient balsam to cover it. Put away out of reach of dust until the benzole has evaporated from the balsam. Then place on a hot plate, apply gentle heat with a spirit-lamp, and

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bake until the balsam is quite hard. Grind down to the required thinness on a hone. Wash well with water, dry, add a few drops of fluid balsam in benzole, and apply a cover-glass.

Metal Specimens.

The preparation of specimens of metal for the microscope involves the greatest care, the principal object being to obtain a perfectly level surface, free from all scratches and marks, with the highest degree of polish. This will be better illustrated by an example.

The student having obtained a sample of metal, the first thing to do is to carefully file or grind the surfaces he wishes to examine. The marks thus made must be taken out with a very smooth file or emery-cloth, gradually diminishing the coarseness of the cloth until he reaches the finest grade of all.

From this stage the polishing must be done on parchment or chamois leather stretched very tightly on wood, the leather being covered with fine crocus-powder or rouge moistened with a little water.

This is the most important stage of the specimen, especially if the metal be very soft, and the student should frequently examine the metal through the microscope—a matter of a few moments only—by clamping it in the new metal-holder recently introduced by Messrs. Watson, as shown on page 38.

It will then be seen that parts stand in very high relief. The object of the leather polishing being to gradually grind away the soft and leave the hard parts, great care must be exercised in doing this.

The specimen is now ready for further treatment—viz., **etching**. The object of this is to further develop the structure, as will be seen from below.

Etching.—This is done by various reagents, the choice of which is mainly a matter of personal opinion, but perhaps the most generally used, and the best for beginners, are

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infusion of liquorice root and tincture of iodine. Very dilute nitric acid and sulphuric acid are also used, but until the student has become thoroughly acquainted with the effects of the above he is not advised to use them.

Before proceeding further it is advisable to give an outline respecting the effects of the reagents, also the construction of the metal.

Steel is viewed as if it were a rock with various constituents in it. There are three principal ones—viz., ferrite, cementite, and pearlite (or sorbite).

Ferrite.—This is iron free from carbon. It retains a very dull polish, and is not stained by iodine or liquorice.

To develop the crystalline structure of ferrite a very dilute solution of nitric acid in alcohol should be used.

Cementite.—This is a very hard substance, and stands in relief after polishing, as above. It is very rarely found in low carbon steels, and is left bright after the polished surface is attacked by iodine.

Pearlite.—This is a very intimate mixture of ferrite and cementite. If the steel has been allowed to cool slowly from a very high temperature, pearlite assumes a well-defined lamellar structure; on the contrary, if the metal has been forged or reheated at a very low temperature, pearlite assumes a granular appearance. It is readily acted upon by iodine or liquorice.

From this it will be seen that steel is made up of (1) ferrite and pearlite, (2) pearlite, (3) pearlite and cementite. Other constituents are found in steel after it has undergone certain treatment, but enough has been said to guide the student to make a commencement.

The reagent can now be applied. This is done by either coating the specimen with some protective varnish, leaving the surface free that is to be acted upon, and immersing the whole in a bath, or a few drops may be applied to the surface, and then carefully spread by means of a glass dipping-rod.

The solution should be allowed to act for, say, twenty

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seconds, then carefully washed in alcohol or methylated spirit, gently rubbing the surface with the little finger, finally washing in water and drying with a very soft piece of linen.

The metal is now examined under the microscope, and it will then be seen if the etching has been sufficient; if not, it should be repeated, as above, for another twenty seconds. The student should do this several times, noting the effect of the reagent each time until he becomes thoroughly acquainted with its properties.

So far we have only dealt with steel, but alloys of tin, copper, etc., are treated in exactly the same way, with the exception that liquorice and iodine are not used. The various acids, ammonia, and caustic potash are then used in weak solution as etching reagents.

With respect to the mounting of the specimens, it will be seen that the new holder does away entirely with the glass slide. It often happens that the lower edge of the metal is left jagged, and may also be broken off at a very sharp angle, necessitating a long delay in filing or grinding, also the metal must not be too thick if a glass slide is used; but, as will be seen, this labour is greatly minimised if the holder be used.

LESSON X.

PREPARING AND MOUNTING ENTOMOLOGICAL SPECIMENS FOR THE MICROSCOPE. MR. ROUSSELET'S METHOD OF PRESERVING ROTIFERA, ETC.

Insects should be killed with chloroform. They are then to be placed in methylated spirit, in which they may remain until required for mounting.

To Prepare a whole Insect for Mounting with Pressure in Canada Balsam.—(1) Transfer from methylated spirit to water, and let it soak for three or four hours to remove spirit.

(2) Place in liq. potassæ—10 per cent. of caustic potash

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in distilled water—until soft. Some specimens will only require a few hours in the potash, others need days, and some even weeks, to soften. In all cases they must be carefully watched and the action of the potash tested. This can be ascertained by pressing on the thorax or chest of the insect with some blunt instrument, such as the head of a pair of curved-pointed forceps.

(3) When soft enough, pour away the potash and add water, which must be changed several times until all the potash is washed away.

(4) Pour away the water and add concentrated acetic acid, and soak for twelve hours, or until it is convenient to go on with the work.

(5) Transfer from acetic acid to water, and soak for about half an hour; then place in a shallow saucer full of water, and with the aid of a needle and a camel's-hair brush spread out the wings, legs, etc. Now take a slide and place it in the water under the insect, lift the slide up carefully so that the insect may be stranded on the surface of the slide with all its parts expanded. Drain off the excess of water, and lay the slide down on a piece of white paper, and with the aid of needles or brushes carefully place all the limbs, wings, antennæ, etc., in their natural positions. Now put a narrow slip of paper on each side of the insect, and carefully lay another slide over it, press it down until the insect is squeezed quite flat, tie the two slides together with a piece of twine, and place them in a jar of methylated spirit for at least twelve hours, or until required.

(6) Remove the glasses from the spirit, carefully separate them, and with a soft camel's-hair brush push the insect off the glass into a saucer of spirit.

(7) Take the insect up on a lifter, and float it on to the surface of a small saucer of clove-oil, and allow it to soak until perfectly clear.

(8) Remove from clove-oil and place in turpentine for a few minutes.

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(9) Mount in Canada balsam as directed for animal and botanical sections.

To Mount an Insect in Canada Balsam without Pressure.—Treat with potash as above, wash in water, and place in acetic acid. Wash away the acid with water, and transfer to a shallow saucer of methylated spirit. Take two needles and lay out the various parts as quickly as possible; if any parts are troublesome, hold them in position until the spirit has fixed them. Now let it soak for an hour, or until required. Remove from spirit, place in clove-oil, and when clear, place in turpentine.

Take a *tin* cell just deep enough for the specimen, and apply a coat of black shellac cement to one side of it. Allow this to nearly dry. Clean and warm a slide over a spirit-lamp; take up the cell in a pair of forceps, and bring the cemented side in contact with the centre of the warmed slide; press on the upper side of the cell, until it adheres firmly to the slide, and put it away to dry. Fill the cell with Canada balsam, and see that it also flows over the upper edge of the cell, so that it may serve as a cement to fasten on the cover. Take the insect from the turpentine on a lifter, put it in the cell, and with needles rearrange the parts if necessary. Put away out of reach of dust for twelve hours to harden the balsam. Place a drop of balsam on one side of the cell. Clean a cover-glass of the same size as the cell, take it up in a pair of forceps, and warm it gently over a spirit-lamp, and bring its edge in contact with the drop of fresh balsam; ease down carefully, so as to avoid air-bubbles, and press on surface of cover with a needle until it rests on the cell all round. Now take a soft brush and some benzole and wash away the exuded balsam; dry with a clean rag, and apply a ring of cement.

To Mount an Insect in Glycerine without Pressure.—Many small, soft insects and their larvæ may be mounted in glycerine while fresh. The larger and harder kinds must be soaked in potash to render them transparent. Make a cell of the required size, and fasten it to a slide with black

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shellac cement, as directed for balsam mounts. Apply a coat of cement to the upper side of the cell, and allow it to nearly dry. Fill the cell with glycerine, and put the insect into it; spread out the wings, legs, etc. Clean and warm a cover-glass, and apply its edge to the cell; press down, and be sure that it adheres to the cement all round. Wash away the excess of glycerine with some water, and dry the slide with a soft cloth. When quite dry, apply a ring of cement, and when this has dried, add another coat of black shellac cement.

The processes described only refer to the study of the external parts of insects; all the soft tissues and internal organs will, of course, have been destroyed by the potash. Soft internal organs must be dissected out of the specimen while under water.

Procure a guttapercha dissecting-dish, lay the insect in it, and secure with pins in the desired position. If the abdominal or thoracic viscera are required, lay the insect on its back; if the nervous system, on its ventral surface. Fill the dish with water, and with a pair of sharp-pointed scissors cut through the chitinous skin on each side of the abdomen, taking care not to cut too deeply so as to injure the internal organs; then with a pair of forceps raise and remove the skin. The organs may now be removed with the aid of a pocket-lens, and washed in distilled water; then stain in borax carmine for several minutes, wash in methylated spirit; then immerse in acidulated alcohol for a few minutes, dehydrate, clear in clove-oil, and mount in Canada balsam.

If desirable to mount the specimen in glycerine, stain as above, then wash away all trace of spirit with water, and mount in glycerine jelly; if the specimen requires a cell, it must be mounted in glycerine.

Salivary glands of cockroaches and crickets, gizzards of beetles, and stings of bees and wasps, may be easily removed in the following way: Place the specimen whole and while quite fresh in water, cover with a piece of paper

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or anything to keep out dust, and let them soak for several days until the smell becomes rather unpleasant; then wash in clean water, hold the insect between the fingers, and with a pair of forceps carefully pull off the head, which should bring with it the œsophagus, salivary glands, and stomach. For stings of wasps and bees proceed as follows: Gently squeeze the abdomen of the specimen between the fingers of the left hand until the sting protrudes, then grip it with a pair of fine forceps, and gently pull it out. If properly done, the poison gland and duct should come away with it. Wash in water, and place it on a slide under a dissecting microscope, and with a fine needle-point draw the stings from their sheath; this is done by putting the needle under the stings at the base of the sheath and carefully drawing it towards the apex. Stain in borax carmine, wash in alcohol, then in acidulated alcohol, and place in water; now lay out on a slide, place another slide over it, tie with thread, and immerse in methylated spirit for several hours; remove from glass, clear in clove-oil, and mount in Canada balsam.

Small insects, such as parasites, may be mounted whole in a cell in glycerine without treatment with potash, so that their internal organs may be seen *in situ*, but they usually require clearing. Take of Calvert's carbolic acid, solid at ordinary temperatures, 2 ounces, melt, and add about ½ a drachm of glycerine to prevent it becoming solid again. Soak the insect in this until transparent; some specimens will only require an hour or two, others a week or more. When clear, make a cell as previously directed with any good shellac cement, and when dry, run on a coat of cement to its upper surface, let this become about half dry, then place in the cell, fill it up with glycerine, and apply a cover-glass, which must be carefully pressed down with a needle-point until it adheres to the cement all round. The slide can then be washed with water to remove all trace of excess of glycerine; put away until all the water has evaporated, then apply a coat of

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shellac cement, and when this has dried, rub away any water-marks that may be left on the slide with a soft cloth, and add another coat of cement.

Wing-cases, legs, heads, and feet of diamond beetles should be mounted in opaque cells in Canada balsam. Take a slide, and with a turn-table run on a disc of black varnish of the required size; allow this to dry thoroughly. Take a piece of black gummed paper and punch out a disc of the same size as that on the slide to which it is to be fastened. Now take a *tin* cell of the required depth—on no account use brass or vulcanite cells; they are affected by the balsam, and the mount will be spoiled—lay the cell on a slide, and apply a coat of cement to its upper surface; allow this to become nearly dry, then take up the cell in a pair of forceps, and bring its cemented surface in contact with the paper disc on the slide, and with the point of the forceps press the cell down until the cement adheres to the paper. Now put away to dry in some place protected from dust. Take the specimen to be mounted, examine it under a microscope, and if dirty, wash in some benzole, and then let it dry again. Now place a small quantity of gum-water in the centre of the cell, and put the specimen into it in the desired position; make sure that it adheres securely to the gum, and put the slide away again until everything is quite dry. Put the slide in a turn-table, and run on a coat of shellac cement to its upper surface, and allow it to become nearly dry; then fill up the cell with Canada balsam, clean, and apply a cover, which must be well pressed into the cement until it adheres firmly; put away for an hour, and then wash away the exuded balsam with a soft brush and some turpentine; dry the slide with a soft rag, and apply a coat of black shellac cement.

Heads of flies having coloured compound eyes, such as Tabanus, lace-wing flies, etc., should be mounted in opaque cells in glycerine. Make the cell in exactly the same way as directed for balsam mounts, but take care that the cell is only just deep enough to take the specimen, as the

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object has to be retained in the centre of the cell by slight pressure on the part of the cover-glass. When the cell is quite dry, apply a coat of shellac cement to its upper surface, and let it nearly dry; then take a brush and some clean water and moisten the inside of the cell. This is done to prevent the formation of air-bubbles, for if glycerine is put into a dry cell, bubbles are sure to give a lot of trouble. Now fill the cell with glycerine and put in the specimen, which should be previously soaked in dilute glycerine for an hour or two, and with a needle place it in the desired position; apply the cover-glass very carefully, so that no air-bubbles may be enclosed, and let it settle down by its own weight until it rests on the surface of the cell; then press it down with a needle-point until securely embedded in the half-dried cement, and set aside for an hour or two to dry. The exuded glycerine may then be washed away by holding the slide under a water-tap. When all trace of glycerine is removed, dry the slide with a soft cloth, and apply a coat of black shellac enamel.

Heads of large insects may be secured in the centre of the cell in the following way: Take a fine needle, thread it with a hair, and run it through the specimen. Unthread the needle, take up each end of the hair with the object suspended and stretch it across the cell so that it may be embedded in the cement on each side. Now apply a cover-glass, press it down until securely fixed, and if the specimen is not in the middle of the cell, adjust it by pulling on the hair on one side. Put away to dry, cut off the ends of the hair close to the edge of the cell, wash away excess of glycerine, dry, and apply a coat of shellac enamel.

PRESERVING AND MOUNTING ROTIFERA.*

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There are few observers of pond life who have not felt a keen desire to preserve and keep these small highly-

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organized sparks of life instead of letting them die and disappear in a few days. For a close study of this group, well-preserved type specimens are of the greatest possible assistance and importance, and if such had existed formerly much confusion and inexactitude in their description and classification would have been avoided, particularly in the giving of three or four different names to the same species, which causes so much trouble to the student.

The total absence of type specimens of rotifers to refer to when required, originally led to an attempt on the part of Mr. C. F. Rousselet to produce them, and it is now over ten years since the first successful experiments at preserving them in a fully extended and natural state were made. His method, although so simple now, took fully three years to work out until the right and most suitable narcotic, fixing agent, and preserving fluid were found. By the use of suitable fixing agents not only the external shape of rotifers can be preserved, but also all the internal structure to the minutest anatomical details, such as the striated muscle fibres, nerve threads, vibratile tags or flame cells, sense hairs, cilia, etc., and frequently important details can be more readily observed than in the living animal.

Narcotizing. — As is well known, no killing agent is sufficiently rapid to prevent the complete retraction of rotifers, and few other animals can contract into such a shapeless mass when we attempt to kill them by ordinary means, such as poisons, alcohol, heat, etc. It is, therefore, necessary to use first a suitable narcotic, which has been discovered in hydro-chlorate of cocaine. As a result of many trials, the best solution for most rotifers has been found to be the following mixture:

2 per cent. solution of hydrochlorate of cocaine, 3 parts;

Alcohol (or methylated spirit), 1 part;

Water, 6 parts.

Another narcotic which is also very suitable for rotifers

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is a 1 per cent. watery solution of hydrochloride of eucaine, recommended by Mr. G. T. Harris, for infusoria and other animals. These narcotics, even so dilute, are not to be used pure, as they would cause the rotifers to contract at once and not expand again. The principle to be followed throughout is to use the narcotic so weak that the animals will not mind it at first, but continue to expand or swim about freely. After a short time its effect will make itself felt on their nervous system, and then some more of the narcotic may be added, until complete narcotization is produced, or until the animals can be killed without contractings.

But before the operation of narcotizing is begun, it is very necessary to isolate the rotifers in perfectly clean water. The best way is to pick them up under a dissecting microscope by means of a very finely drawn-out pipette, having a funnel-shaped enlargement at the other end, which is covered with an elastic membrane. This pipette forms a most delicate syphon, by means of which any selected rotifer can readily be taken up with the least quantity of water, and transferred to another trough or watch-glass full of clean water. This preliminary precaution is necessary, because particles of dirt in the water readily attach themselves to the cilia of dead rotifers, rendering them unsightly under the microscope. Another advisable precaution is to separate the different species, because most species require a slightly different treatment, and because the small species too readily adhere to the cilia of the large species.

Having then isolated a number of free-swimming rotifers in a watch-glass half full of perfectly clean water, one drop of one of the above narcotics is added and well mixed. After five or ten minutes, if the animals continue to swim about freely, another drop is added, and so on until the effect of the narcotic becomes visible, and until the motion of the cilia, or the movements of the animals slacken or almost cease, when they are ready for killing. The effect

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of the narcotic varies very much with different species; some are most sensitive to it, whilst others can stand a considerable quantity for a long time.

Killing and Fixing.—Some practice and patience are certainly required to find out the right time to kill the different species; no general rule can be given, as the time may vary from fifteen minutes to several hours. It is very essential, however, that the rotifers be still living when the killing fluid is added to prevent post-mortem changes in the tissues which begin at once on the death of the animals.

For killing and fixing several fluids are suitable—namely, ¼ per cent. osmic acid, or Flemming's chromo-aceto-osmic fluid, or Hermann's platino-aceto-osmic mixture. On the whole, I now prefer the last-named, which gives a finer fixation of the cellular elements of the tissues and does not stain them so much. It may be explained that the term 'fixing' implies rapid killing and at the same time hardening of the tissues to such an extent as to render them unalterable by washing and subsequent treatment with preserving fluids. Proper fixation is very essential, as no good preservation can be obtained without it.

When the rotifers are narcotized and ready for killing, a single drop of one of the above fixatives is added, and mixed with the water in the watch-glass. A few minutes is sufficient for fixing small creatures like these, and then they must be removed again by means of the pipette to several changes of clean water to get rid of the acid, otherwise they will become more or less blackened. When dealing with marine rotifers, sea water must be used for washing out, for the difference in density between fresh and sea water is sufficient to cause swelling by osmosis, and the consequent spoiling of the specimen. After thoroughly washing, the rotifers are transferred to a preserving fluid, the density of which does not materially differ from that of water. The best preserving fluid found so far is a 2½ per cent. solution of formalin, which is made by mixing

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2½ c.c. of the commercial 40 per cent. formaldehyde with 37½ c.c. of water, and then filtering.

The above are general directions according to which the great majority of rotifers can be preserved. When under the narcotic, the animals must be watched until it is seen that they can swim but feebly, when, as a rule, they will be ready for killing. If they contract and do not expand again, it is a proof that the narcotic used is too strong, and it must be further diluted. The whole method undoubtedly requires great care, and is a delicate operation, which must be performed under some kind of dissecting microscope, but by following the directions here given, and with some perseverance, anyone can learn to prepare a large number of species of rotifers. I would advise that a beginning should be made with some such forms as *Brachionus*, *Anuræa*, *Synchœta*, *Asplanchna*, *Hydatina*, *Triarthra*, and *Polyarthra*, which are easy, and, moreover, occur, and can, as a rule, be collected in large numbers. A few genera, however, are exceptionally difficult. These are *Stephanoceros*, *Floscules*, *Philodina*, *Rotifera*, and *Adineta*, and it will be better to leave these until considerable experience in dealing with the others has been acquired.

It will have been noticed that the rotifers must always remain submerged in a watery fluid, and be transferred in a drop by means of the pipette. Fluids of lesser density than water, such as alcohol, as well as fluids of greater density, such as glycerine, are unsuitable because they set up strong diffusion-currents by osmosis, which cause the animals either to swell or to shrivel up completely.

Some species of rotifers, such as *Triarthra*, *Polyarthra*, *Pedalion*, *Mastigocerca*, etc., have an outer surface which is strongly water-repellent, and when these come in contact with the surface film of the fluid even for an instant it is most difficult to submerge them again, and, as a rule, they are lost or spoiled.

Having then successfully narcotized, killed, and fixed the rotifers fully extended, and finally transferred them into

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2½ per cent. formalin, the animals may be kept in little bottles, or mounted in the same fluid on micro-slides, either in excavated cells or shallow cement cells.

Mounting.—To mount on a slide, place a drop of the formalin solution in the cell, then transfer the prepared rotifers into this drop with the pipette, and examine under the dissecting microscope to see that no particle of foreign matter has been introduced. Then place another drop of the fluid on the slide by the side of the cell, lower the cleaned cover-glass on that drop, and push the cover cautiously and gradually over the cavity. The superabundant fluid is removed with blotting-paper, and the slide closed by tipping damar-gold size cement all round the edge with a fine brush.

The permanent closing of these cells has been a matter of very considerable difficulty. As the result of the experience gained, it is recommended that the cells be closed first with a coat of a varnish consisting of two-thirds damar in benzole and one-third gold size, then two coats of pure shellac dissolved in alcohol, and finally four to six coats of pure gold size. Each layer of cement must be allowed to dry thoroughly well; three days for each layer is not too long.

By the method described above, Mr. Rousselet has in the course of the last ten years made a collection of over 500 slides containing nearly 300 different species of rotifers, probably the only collection of the kind in existence, which is of the greatest use for the identification of species and for the general study of this interesting class.

Entomostraca should be narcotized with the same solution as used by Mr. Rousselet for *Rotifera*, then killed with a ¼ per cent. solution of osmic acid, and mounted in a 2½ per cent, solution of formalin.

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LESSON XI.

CRYSTALS AND POLARISCOPE OBJECTS.

Crystals.—*Method* 1.—Make a strong solution of the material in distilled water, with the aid of heat if necessary, and filter; take up a small quantity of the solution in a dipping-tube, and drop it on a cover-glass. Prepare several covers in this way, and allow some to dry slowly, and evaporate others over a spirit-lamp. When dry, add a drop or two of Canada balsam, and mount in the usual way.

Method 2.—Make a strong solution in distilled water, and add a few drops of gum water or a small piece of gelatine; mix well, and filter. Apply some of the solution to a cover-glass, and allow it to dry slowly in a place protected from dust. Mount in Canada balsam.

Method 3.—Place a small piece of the dry crystal on a slide, and apply a cover-glass; warm over a spirit-lamp until fusion results, press the cover down with a needle, and allow the slide to cool. Clean off the exuded material, and finish off with some good cement.

Some crystals are soluble in Canada balsam; in which case, mount in castor-oil.

Crystallize the specimen on the cover-glass; make a thin cell with some shellac cement on a slide, and allow it to become perfectly dry; then apply another coat of cement, and when this has nearly dried, fill the cell with castor-oil. Take up the cover with a pair of forceps, and bring the crystallized surface in contact with the oil, being very careful that no air-bubbles form. Ease it down gently, and when it rests on the cell, give it a press with the point of the forceps; this will squeeze out the excess of oil and embed the edge of the cover in the cement. Put away to dry; wash off the exuded oil with some turpentine, and apply another coat of shellac cement.

The following salts, etc., are easily obtained, and they all give very good results:

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Chloride of barium.*	Sulphate of iron.*	Asparagine.
Chlorate of potash.*	Tartrate of soda.*	Quinidine.
Sulphate of copper.*	Salicine.	Santonine.
Spermaceti (fuse).	Stearine (fuse).	Tartaric acid.

Those marked * are more effective when crystallized in gum or gelatine.

Crystals of Silver.—Clean a cover-glass and fasten it to a slide with the breath; make a 1 per cent. solution of nitrate of silver, and place a drop of it in the centre of the cover-glass. Now add a very small fragment of copper, and put the slide away out of reach of dust until the crystals have formed, and all moisture has evaporated. Then make a shallow opaque cell, and place a small drop of gum water in its centre. Take up the cover with a pair of forceps, crystals uppermost, of course, and drop it into the cell; now take a needle-point, and carefully press on the cover-glass between the crystals, until it lies quite flat, and air-bubbles, if any, have exuded. Put the slide away again until the gum has dried. Now put the slide into a turn-table; run on a coat of shellac cement to the upper surface of the cell. Allow this to become half dry, and then apply a cover-glass.

The following specimens from the vegetable kingdom make fine polariscope objects: Starches, hairs, scales from leaves, cotton and silk fibres, cuticles of leaves, and longitudinal and transverse sections of stems.

Starches can be obtained from most vegetable substances by scraping the cut surface with a knife. Place the scrapings in a bottle of water and shake well; then strain through muslin of sufficiently fine texture to allow the starch to pass, but to retain the fibres. Now put the strained material into a bottle, shake it up, and then allow to settle; the starch will fall to the bottom of the bottle in a few minutes. Then pour off the water; add some more, and repeat the process until all trace of cellular tissue is removed. When the starch is quite clean, take up a little in a dipping-tube; apply a drop to a clean cover. See that it spreads evenly

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all over the surface of the cover, and put away, protected from dust, until quite dry; then add a drop of Canada balsam, and mount in the ordinary way.

Starches may also be mounted in glycerine jelly (see Lesson VIII.), but they do not polarize so well as the balsam preparations.

Sections of Starch-bearing Tissues.—The stems, roots, and bulbs must be hardened in methylated spirit for a week; then make transverse or longitudinal sections. Dehydrate in methylated spirit, clear in clove-oil, and mount in Canada balsam.

Cuticles containing Raphides.—The most common are taken from the following bulbs: garlic, onion, lily, hyacinth. Strip off the cuticle from the fresh specimen; dehydrate in methylated spirit, clear in clove-oil, and mount in Canada balsam.

Cuticles of Leaves. — Cut up the leaf into small pieces, and soak in water until rotten; the cuticles can then be separated, washed in water, dehydrated in methylated spirit, cleared in clove-oil, and mounted in Canada balsam.

Cotton, Hemp, Wool, Silk, Flax, etc.—Place the fibres in methylated spirit to dehydrate; then clear in clove-oil, and place a little on a slide. Separate the fibres from each other with needle-points; apply a few drops of Canada balsam and a cover-glass.

Scales of Leaves.—Scrape the leaf with a knife, and put the scrapings into a bottle of turpentine, and soak until all trace of air has disappeared from the scales; then pour off the turpentine. Take up a little of the scales on the point of a penknife, and mount them in Canada balsam in the ordinary way. Some leaf-scales are very difficult to deprive of air; in fact, it is impossible to get them quite free.

The following animal tissues make good polariscope objects: fish scales, palates of molluscæ, sections of hairs and quills, horns and hoofs, whalebone, claws of dogs, cats, and fowls, decalcified bones, muscular tissues.

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Fish Scales.—Scrape the fish from the head towards the tail; if scraped the other way, nearly all the scales will be injured. Place the scrapings in a bottle of water, shake well, pour off the water, and repeat the process until quite clean. Examine with a microscope, and if you find that the scales are not clean, pour off the water, add liq. potassæ, and soak for an hour or two; then wash away the potash with repeated changes of water, dehydrate in methylated spirit, clear in clove-oil, and mount in Canada balsam.

Sometimes fish scales buckle up in spirit, and they will not lie flat. When this happens, put them into water again, and soak a little while; then place them on a slide, and put another slide over them, press down until quite flat, and tie the two glasses together with twine, and place them in a vessel of methylated spirit to dehydrate under pressure. This method will answer for all tissues that have a tendency to twist during the process of dehydration.

Palates.—Dissect out, and soak in liq. potassæ for a few days. Wash well in water, spread out on a slide; put a piece of paper on each side of it to prevent crushing, and place another slide over all in the same way as directed for insects; tie the glasses together with string, and place in methylated spirit for an hour or two. Then remove the palate from the glasses, and place it in clove-oil until clear. Mount in Canada balsam.

Sometimes it is very difficult to dissect out the palates from small snails. This process answers just as well: Cut off the head of the animal, being careful that you remove the buccal mass with it, and place in liq. potassæ for a few days; this will destroy all the soft tissues, but not the palate or radula. Wash away the potash with repeated changes of water, and proceed as directed above.

Sections of hairs and quills may sometimes be cut after soaking for a few days in methylated spirit; but some of the larger kinds, such as the whisker of walrus, will require softening in potash. Place in liq. potassæ for a few

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hours or days, in accordance with the consistency of the tissue. When soft enough, wash away the potash with water, and place in methylated spirit, in which they may be preserved until required. Then make transverse and longitudinal sections, dehydrate in methylated spirit, clear in clove-oil, and mount in Canada balsam.

Small Fine Hairs.—Cut off a number of hairs, tie them up into a bundle with some cotton, and soak for a few minutes in warm water. Make up a strong solution of gelatine in water, and transfer the bundle of hairs to it, and soak it for several hours in a hot-water bath until the gelatine has penetrated to the centre of the bundle. Remove from the gelatine on the point of a needle, and hold it exposed to the air until the gelatine has cooled; then push them from off the needle into a bottle of methylated spirit, and soak for an hour or two to complete the hardening. Embed in carrot, put in a microtome, and cut transverse sections, and as they are cut place them in methylated spirit to dehydrate; then clear in clove-oil, and mount in Canada balsam.

Horns, hoofs, whalebone, and claws all require steeping in liq. potassæ until soft; they are then to be washed in water, and preserved in methylated spirit until required. Embed in carrot, place in a well microtome, make transverse and longitudinal sections, dehydrate in methylated spirit, clear in clove-oil, and mount in Canada balsam.

Decalcified Bones (see Lesson I.).—Embed in carrot, make transverse and longitudinal sections, dehydrate in methylated spirit, clear in clove-oil, and mount in Canada balsam.

Muscular Fibres.—Take the tongue of a cat, harden it in methylated spirit for a week or ten days; then embed in carrot, and make transverse or longitudinal sections, dehydrate, clear in clove-oil, and mount in Canada balsam.

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LESSON XII.

CLEANING AND MOUNTING DIATOMS, POLYCYSTINA, AND FORAMINIFERA.

To Clean Diatoms growing upon Algæ or Shells.—Place the Algæ or shells in a basin, cover them with water, add hydrochloric acid, and stir until effervescence results; add more acid little by little, until effervescence ceases, stirring from time to time. Now strain through net of sufficiently fine texture to allow the diatoms to pass, but to retain the débris. Allow the strained fluid to settle down, pour off the acid water, and place the deposit in a large test-tube. Add pure hydrochloric acid, and boil for twenty minutes; add some pure nitric acid, and boil again for twenty minutes, and, while boiling, add some crystals of chlorate of potash until complete bleaching results. Remove all trace of acid or alkali by washing in water, and examine the forms under the microscope. If clean, bottle them up in distilled water for future mounting. If, as is sometimes the case, there has been animal matter present which has not been removed, boil in pure sulphuric acid for a few minutes. Wash away all trace of acid before bottling the diatoms in distilled water.

To Clean Fossil Diatomaceous Deposits.—Break the deposit up into small pieces, and place them in a large test-tube in a moderately strong solution of bicarbonate of soda, and boil gently for two hours, the disintegrated portions being from time to time poured off into a beaker and the boiling in soda continued until all the deposit has broken up. The alkaline solution must then be washed away, and the diatoms boiled for a short time in nitric acid, and when sufficiently clean wash away the acid in repeated changes of water, and bottle up the diatoms in distilled water.

To Clean Living Diatoms.—Remove all dirt or salt by washing well in water; shake well, and allow the diatoms

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to settle before pouring off the water. In this way all soluble impurities can be removed. When the water remains clear, pour it off, leaving the diatoms as nearly dry as possible, and cover them with strong alcohol, which will extract the endochrome; change the alcohol daily until it ceases to be tinged with green; then wash away the alcohol with water, pour off the water, and place the diatoms in a platinum capsule and heat them to a dull red over a spirit-lamp. This will separate the frustules into single valves, and finish the cleaning of the diatoms, and they may then be bottled up in distilled water.

To Clean Polycystina.—The polycystinous earth should be broken into small pieces and boiled for several hours in a strong solution of common washing soda, the disintegrated matter being from time to time poured off into a vessel, and the boiling in soda continued until all the earth is broken up. Wash the disintegrated matter in water several times to remove the soda, allow the polycystina to settle down, and pour off the water and place the forms in a test-tube; add some nitric acid, and boil for twenty minutes. Remove all trace of acid with water, and bottle up in distilled water.

To Clean Foraminifera.—All mud must be got rid of by repeated washing in water. Then boil the forms in a strong solution of bicarbonate of soda for an hour or two. When clean, wash away the soda, and bottle in distilled water.

To Mount Diatoms in Canada Balsam (*Unselected Slides*).—The diatoms are to be taken out of the bottle with a dipping-tube, and should be allowed to fall upon a clean cover-glass. The fall of the drop causes the forms to spread evenly over the cover. It should then be dried slowly over a spirit-lamp. When dry a small drop of Canada balsam is to be applied, and the slide put away out of reach of dust to dry for twelve hours. Now place on a hot plate, and apply gentle heat from a spirit-lamp for about ten minutes. Allow it to cool. Take the cover

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up with a pair of forceps, and bring its balsamed surface in contact with the centre of a warmed slide. The balsam should then run to a neat bevelled edge all round the cover; should it not do so, warm the slide a little more until it does.

Unselected Polycystina.—Take the forms from the bottle with a glass tube, and spread them on a slide; dry them over a spirit-lamp. Now clean a cover-glass, fasten it to a slip with your breath, and place a drop or two of balsam on it; take up some of the polycystina on the point of a knife and place them in the balsam; stir them well up with a needle and put away for twelve hours. Bake over a spirit-lamp for ten minutes, and while warm stir up again gently with a needle, and spread the forms evenly over the cover. Warm a glass slide, and proceed as directed for unselected diatoms.

Unselected Polycystina as Opaque Objects.—Dry some polycystina on a slide, then take a platinum capsule, put the dried material into it, and heat over a spirit-lamp to a dull red. Clean a cover-glass, fasten it to a slide with your breath, and apply a few drops of balsam. Take up some of the dried forms, put them into the balsam, and stir up with a needle until they are evenly spread over the cover; put away out of reach of dust for twelve hours, so that the air may escape from the forms. Now place on a hot plate and apply gentle heat for ten to fifteen minutes to bake the balsam. Clean another cover-glass, add a drop or two of balsam to the hardened balsam, and apply the second cover-glass; warm again, and with a needle press gently on the upper cover until it lies perfectly flat; then allow to cool, apply a coat of black shellac cement all over one side of the upper cover, and put away to dry. In the meantime take a slip, put it in a turn-table, and run on a disc of black varnish of the same size as the cover; let this dry, then add a drop of strong gum or glue; take up the covers with a pair of forceps, and put the blackened side into the glue; press down with a needle until the glue

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spreads evenly under the cover, and put away to dry. When dry, finish off with a coat of black cement.

Selected Diatoms and Polycystina.—Take an ounce of distilled water, add 6 or 8 drops of ordinary gum water, and filter. Clean a cover-glass, and place a drop of the diluted gum upon it; put away to dry.

Spread the diatoms or polycystina on a slip, and dry them over a spirit-lamp. Select the desired forms with a fine brush or bristle, and breathe upon the gummed surface of the cover, and place the forms upon it. When dry, apply a drop of balsam, and put away out of reach of dust for twelve hours. Bake and finish as directed for unselected slides.

In mounting selected polycystina, they must be between two covers; if on a single cover, the forms would be upside down when the cover was reversed. If a transparent mount is desired, the two covers can be fastened to the slide with a drop of balsam. If opaque, the forms must be burnt, and one side must be blackened; in other respects proceed exactly as you would for unselected opaque mounts.

Polycystina may also be mounted in a dry opaque cell. Take a slide, run on a disc of black varnish, and when this has dried, fasten a disc of black gummed paper over it. Then take a shallow cell, apply a coat of cement to one side of it, and let it nearly dry; then fasten to the paper disc, and put away to dry. Apply a little dilute gum water to the bottom of the cell, select the specimens, and put them into the gum; if they do not adhere, breathe on the surface of the gum. When all are arranged, put the slide away until everything is quite dry; then add a coat of cement to the upper side of the cell, let it nearly dry, and then apply the cover-glass.

Foraminifera—Unselected Transparent Mounts.—Dry the forms on a slide with the aid of gentle heat, and scrape them off into a bottle of turpentine, in which they must soak until all trace of air has disappeared. Then clean a cover-glass, fasten it to a slide with condensed

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breath, and apply a few drops of balsam. Pour off the turpentine from the foraminifera, take up some of the forms on the point of a penknife, and put them into the balsam on the cover; stir up with a needle until spread evenly, then put away for twelve hours. Bake gently for ten minutes on a hot plate, cool, apply a drop of fluid balsam, warm a slide over a spirit-lamp, take the cover up in a pair of forceps, and bring the drop of fluid balsam in contact with the centre of the slide, ease down carefully, and press on the upper surface of the cover with a needle-point until it lies quite flat; or if the forms are very delicate, warm the slide again gently until the cover settles down by its own weight. Allow the slide to cool, then clean away exuded balsam with methylated spirit, and apply a coat of cement.

Foraminifera—Opaque Mounts.—Proceed in exactly the same way as directed for mounting dry opaque polycystina; but if the specimens are unselected, gum the bottom of the cell, dry the forms on a slide, and spread a quantity of them all over the surface of the cell. Let the gum dry, then shake out all that have not adhered, apply a coat of cement to the upper side of the cell, and when this has nearly dried, apply a cover-glass.

Spicules of Gorgonia or Sea-fan.—Boil in liq. potassæ until all the material has broken up, then wash away the potash with repeated changes of water, allowing the spicules to settle to the bottom of the tube between each washing. When cleaned, preserve in a bottle of dilute spirit. Proceed with the mounting in exactly the same way as directed for transparent unselected polycystina.

Spicules of Alcionium.—Proceed as above.

Sponges.

1. **To show Cell Structure, Flagellated Cells, etc.**—Fresh specimens of the calcareous forms — Sycon, for example—should be fixed with osmic acid 1 per cent.

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solution, washed in distilled water, and placed in absolute alcohol for twelve hours; then soak in absolute alcohol and ether for a few hours, infiltrate, and embed in celloidin. Cut sections in a microtome. Place sections in absolute alcohol for about three minutes, clear in oil of origanum, and mount in Canada balsam.

If preferred, sections of sponges may be mounted in glycerine jelly, but they must be soaked in water for a little while before they can go into the jelly.

2. **The Skeleton**—(*a*) *Horny Sponges.*—Boil in liq. potassæ, then wash the spicules well in water, and mount in glycerine jelly or Canada balsam.

(*b*) *Calcareous.*—Dehydrate small forms in alcohol, clear in clove-oil, and mount in Canada balsam in a cell; or separate the spicules by boiling in liq. potassæ, wash in water, and mount in Canada balsam or glycerine jelly.

(*c*) *Siliceous.*—Boil in nitric acid, wash well in water, dehydrate, clear, and mount in Canada balsam.

For the types in which siliceous spicules are embedded in horny material, boil in liq. potassæ for a few minutes to disintegrate the tissues, then in nitric acid to clean the spicules, wash well in water, and mount in Canada balsam.

Sections of Sponges.—Harden in methylated spirit, and transfer to equal parts of ether and absolute alcohol for several hours. Then place in a thin solution of celloidin for a day or two, transfer to a thicker solution of celloidin, and soak again for a few hours. Remove from the celloidin on the point of a needle, and hold exposed to the air for a few minutes to allow the celloidin to set around the specimen; then push it off the needle into a bottle of methylated spirit, and soak for a few hours to complete the hardening. Embed in carrot, place in a well microtome, and make the sections. Dehydrate in methylated spirit, clear in oil of bergamot, and mount in Canada balsam.

Sometimes sponge sections are rendered too transparent by mounting in balsam. In such cases, mount in glycerine

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jelly, but be careful to wash away all trace of alcohol before they go into the jelly.

COLLECTING AND PREPARING FORAMINIFERA.*

MR. A. EARLAND'S METHOD (*By permission.*)

The foraminifera, in spite of their beauty, the important part which they have played in the building up of our earth, and the many interesting features of their life-history, have not met with so much favour among microscopists as many groups of far less importance. This comparative neglect is largely due to mistaken ideas as to the difficulty of obtaining and preparing suitable material, and it is proposed to show, so far as possible within brief limits, that the collection of material is within the reach of every visitor to the seaside, and that the subsequent preparation presents no unusual difficulty to the microscopist.

The chief sources from which foraminifera may be obtained are:

1. Dredged material, including anchor muds and sands.
2. Shore gatherings made between tide marks.
3. Sands, clays, and limestones of various geological ages, especially from cretaceous and tertiary deposits.

Probably very few readers will have the opportunity of dredging for material; and anchor muds, which often contain an abundance of shallow water forms, are rarely obtainable, owing to the strange reluctance of seamen to lend themselves to the collection of scientific material; but the method of preparation for materials of this class is essentially the same as that for shore gatherings.

The apparatus required by the shore-collector is of the simplest character, and consists of a scraper for removing the surface film of sand, which alone contains foraminifera, a spoon for scraping material from ripple marks and depressions, and a metal box or canvas bag to contain the

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gathering. The best scraper is a thin plate of celloidin, such as a 'Photographic' film, as the thinness and flexibility of this material enables the collector to make his scraping with less admixture of sand than is possible with the glass or metal slip usually recommended for use.

Thus equipped, the collector sallies forth between the tides. Probably everyone has noticed when at the seaside the white lines which run along the sands parallel with the retreating tide. A pocket lens shows that the white material consists of the minute shells of foraminifera, of which some are of a lustrous white colour, due to the comparative abundance of the *Miliolidæ*—a family of common occurrence in shore-gatherings, characterized by opaque shells of a milky white or 'porcellanous' texture—while others are more or less glassy and transparent. These 'hyaline' forms are much less noticeable to the naked eye. They are mixed in varying proportions with fragments of shell substance—ostracode shells, cinders, and the lighter débris of the shore—and their presence in these lines is due to the separating action of the water, which on a smaller scale we shall later on employ in the cleaning of our collected material. The rocking action of the wave on the extreme edge of the ebbing tide keeps these shells and fragments of light specific gravity in suspension until after the heavier sand-grains have subsided, and so they are left behind in the ripple marks and depressions of the sand. Sometimes a local eddy of the tide, produced by the neighbourhood of a projecting rock, or of groins and piers, causes the material to be gathered together in large quantities, which show as extensive white patches on the sand, and prove a real gold mine to the collector, who will then obtain more material in half an hour than he could gather in several days from the ripple marks.

The collector must not conclude that there are no foraminifera present because there are no white patches to be seen, but, remembering the way in which these patches are

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formed of the lighter débris of the shore, must look for foraminifera wherever he observes that such débris has been deposited.

On every coast, at intervals of varying distance, there are spots which appear to be the foci of the local tides and currents, and here the material will be found in the greatest abundance. These points will soon be discovered, and may be worked at every tide, but they vary continually with the set of the tide and wind, so that a spot which has proved rich may be quite bare the next year. Thus, in October, 1896, Bognor — always a rich collecting-ground —had its richest point to the west of the pier; while in September, 1901, there was very little material obtainable except at Felpham, two miles to the east, where the beach was thick with débris.

Having found the material, the collection is quite an easy matter. With the celluloid scraper at an angle of 60°, the thin surface film of foraminifera and débris is easily scraped into a heap and transferred to the box or bag. Great care must be exercised not to dig down into the sand, for nothing but a heavy bag will result from this, the foraminifera being confined to the surface layer. The material thus collected may be either cleaned at once, or, after being slowly dried—avoiding great heat—may be packed away in bottles for a more convenient period.

The apparatus required for the cleaning and preparation of the dried material is simple and inexpensive, and, if desired, much of it may be easily improvised. The most necessary articles are a photographic developing-dish of china, quarter or half plate size according to fancy, sieves of different sizes and materials according to the collector's pocket, a cylindrical glass jar with a lip, and without any neck or constriction at the top, and a retort stand or tripod, made of an iron ring riveted on three legs.

The sieves can be made by any coppersmith, and it is very convenient to have a series of varying degrees of coarseness; but for the beginner, two sieves of 40 and

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120 meshes to the inch respectively will be sufficient. The writer's sieves are of copper, 4 inches high, 4 inches diameter at top, sloping to 3 inches diameter at the bottom. A smaller size, made of telescope-tubing $1\frac{1}{2}$ inches in diameter and 1 inch deep, is very useful for washing small gatherings. Zinc, which is cheaper than copper, can be used for the sieves.

The wire gauze, which can be obtained from any large ironmonger, varies in price according to the number of meshes to the inch, ranging from a few pence per square foot to four shillings for the finest obtainable, which has 120 meshes to the inch, the diameter of each aperture being about $\frac{1}{200}$ inch. If a finer sieve than this is required, as it sometimes may be, the size of the aperture may be reduced by silver-plating the gauze, or, preferably, by the use of silk bolting cloth, which may be obtained up to 200 meshes to the inch. The wire gauze must be strained tightly over the sieve and soldered neatly to the edge, so that there is no ledge of solder inside to retain unwashed material. If silk is used, a sieve must be made without a bottom, and having a turned-back edge at the lower end, so that the silk may be strained across and secured with string or a rubber band. The most useful sizes for a series of sieves are, in my opinion, 12, 20, 40, 80, 120 and 150 (silk) meshes to the inch.

Before cleaning the material it must be slowly and thoroughly dried. It should then be passed through the twelve-mesh sieve to remove all the coarse débris, stones, shells, cinders, etc. None of the British shore species, except parasitic forms, will be found in this coarse residuum, but it should be looked over with a pocket lens for these or for abnormally large specimens. In some dredged materials and in tropical gatherings, however, this coarse residuum will be found to be full of foraminifera.

The material which has passed through the twelve-mesh sieve consists of foraminifera mixed with other light débris and a considerable quantity of sand, and the collector

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must now proceed to eliminate the whole, or nearly the whole, of the sand and as much as possible of the other débris by means of two operations—'floating' and 'rocking.' If the quantity of material to be operated upon is small, it may be treated off-hand, but if there is much, it is well to sift it out into varying degrees of fineness by passing it through a series of sieves. This will simplify the floating operations by insuring that the particles are approximately of similar weight.

The floating operations must be performed at a sink, and, if possible, in daylight, the process being more uncertain by artificial light. The finest sieve (120 wire or 150 silk) is thoroughly wetted and rested on the tripod. The glass jar is then filled with water nearly to the brim, and a few spoonfuls of sand slowly poured into it. If the material is coarse the sand sinks instantly, and in the course of a few seconds most of the foraminifera follow suit. By holding the jar to the light the course of the falling particles can be followed, and at the proper moment a sudden tilt empties the whole of the water and most of the foraminifera into the sieve, the sand and a few of the heaviest 'forams' being left in the jar. The purity of the material in the sieve, which is usually called 'floatings,' will depend upon the skill and judgment of the operator, and is largely a matter of practice. The residuum in the jar must be washed out into a basin for further treatment, and the operation repeated with more sand and water until the whole of the gathering has been treated. The time allowed for subsidence will vary with the fineness of the sand, so that in the case of the finest siftings nearly a minute may be required. The actual time can only be determined by watching the falling material in a strong light.

In the case of very fine sand, the tension of the surface film of water is so great that the sand grains float almost as readily as the foraminifera. This difficulty may be overcome by shaking up the contents of the jar, covering up the top with one's hand while so doing.

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The residuum, which had been set aside in a jar, may now be treated by the 'rocking' process for the separation of the remaining foraminifera. Taking the photographic developing dish, or a tin tray may be used as a substitute, enough of the residuum is placed in it to cover the bottom to a depth of about $\frac{1}{4}$ inch, and covered with about $\frac{3}{4}$ inch of water. If the dish is then rocked with a combined up and down and circular motion, the foraminifera will rise in suspension in the water, and by a little careful manipulation may be gathered in one corner of the dish. A sudden tilt will then empty them with the water into a sieve. The operation should be repeated with two or three lots of water, and the material left in the dish will then be found to consist almost entirely of sand. The material left in this second sieve, known as 'washings,' is not so pure as the 'floatings,' for it contains a large percentage of broken forms and shell fragments, coal-dust, and other débris. It may be further purified, if desired, by being dried and 'floated' once or twice in the glass jar.

If the floatings thus obtained contain much animal or vegetable matter, as is sometimes the case, it is advisable to boil them in a solution of caustic potash. This will not damage the foraminifera so long as the boiling is not carried on too long, and it effectually removes the animal matter, which otherwise would encourage fungoid growths.

The processes already described are intended for recent *sandy* gatherings. When the material is in the form of dredged mud, it is first necessary to get rid of the finest particles of this mud, for if the water is turbid it becomes very difficult to judge the right moment for separating the floating forams. The mud should be broken up into small lumps, about an inch cube, and slowly but thoroughly dried. It is then placed in a basin and covered with water, which rapidly breaks it up into a fine mud. Such specimens as may be observed floating on the surface of the water may be easily removed by means of cigarette-

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papers, which are placed on the surface of the water. The forams adhere to the papers, which are then carefully lifted off and dried, the specimens being then brushed off into a tube. Many delicate forms, which would almost certainly be broken in the subsequent processes, may thus be obtained in a perfect state.

The mud remaining in the basin is then washed, a spoonful at a time, by placing it in a sieve of fine silk gauze, through which a gentle stream of water from the tap is kept running until all the fine particles have been removed. The muddy water should be allowed to settle in a bath, and the solid residuum scraped out and thrown away. The sandy residuum left in the sieve should then be thoroughly dried, and is then ready for examination under the microscope, or, if desired, it may be further purified by the floating and rocking processes already described.

Foraminifera occur in marine fossil deposits of all geological ages, from the Cambrian to post-tertiary, but they are, as a rule, of sparing occurrence until we reach the cretaceous period. The harder chalks and limestones can only be studied by means of thin sections, but the softer chalks, shales, and clays may be broken up by drying the material in small pieces and washing it over a fine sieve in the manner just described. Floatings are seldom procurable from fossil deposits, owing to the weight of the specimens, which are generally more or less infiltrated with pyrites or other mineral matter.

Some chalks and shales which resist the disintegrating action of water after being dried may be broken up by the action of a crystallizing salt, which has been absorbed in a fluid state. Acetate of soda has the most rapid action, but very fair results may be obtained with common washing soda. The material, after being broken up into small pellets, is dropped into a boiling saturated solution of the salt, and kept at this temperature for a short time to allow of penetration. The salt is then allowed to cool, and in

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cooling crystallizes, the formation of the crystals breaking up the outer layer of the material. On being warmed, the soda dissolves again in its own water of crystallization, and the crystallization is repeated over and over again until the lumps are broken up. The resulting mud is then washed in the ordinary way.

The best foraminifera from the chalk are those obtained from the interior cavities of hollow flints. They are often in the most perfect state of preservation, and the chalk in these cavities being of a powdery nature, they are very easily cleaned.

The cleaned material should be sifted into varying degrees of fineness, and each grade kept separately in a tightly corked tube, noted with locality, date, and any details as to the species contained in it, which may be likely to be useful for future purposes of reference. If the material has been properly cleaned and dried it can be kept unaltered for an indefinite period, but if put away damp fungoid growth will quickly set in. This can be destroyed, and the material sterilized, by a prolonged soaking in spirit, the material being afterwards dried once more.

To examine the material under the microscope, a picking-out tray will be necessary. This is made by covering a slip of card with coarse black-ribbed silk, the ribs running longitudinally along the slip. A thin wooden ledge must be glued round three sides of the slip to prevent the forams rolling off when the stage of the microscope is inclined at an angle. The material is sprinkled over the slip, and the ridges of silk keep the forams from rolling about. The specimens required can then be easily selected by means of a fine sable brush, moistened by drawing it between the lips, and transferred to a prepared cell or slip.

The best fixative for mounting foraminifera is gum tragacanth, which is almost invisible when dry, being quite devoid of the objectionable glaze which characterizes gum arabic. It is also much less subject to variations of

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moisture than gum arabic, which alternately contracts and expands with changes of weather, and often fractures delicate forms. Powdered gum tragacanth should be used in the preparation of the mucilage. Put a small quantity of the powdered gum in a bottle with sufficient spirit of wine to just cover it. Add a small crystal of thymol or a few drops of clove-oil, or oil of cassia, as an antiseptic, then fill the bottle with distilled water and set it by for some hours. The gum will form a thick mucilage, and may be used of varying thicknesses according to the size of the foraminifera. For most forms it should be of about the consistency of cream, and it may be used liberally in mounting, as it shrinks very much in drying.

The same gum diluted to a watery consistency can be used as a fixative for foraminifera mounted in balsam. If the slide is thoroughly dried before the balsam is added the gum becomes quite invisible.

For very large and heavy foraminifera, seccotine or some other liquid glue may be used with advantage, gum not being of sufficient strength to hold them safely.

Many fossil foraminifera and recent forms from some localities have the internal chambers filled with mineral infiltrations, either glauconite or pyrites. These internal casts reproduce more or less perfectly the shape of the sarcode body of the animal. They may be obtained by decalcifying the specimens with very dilute nitric acid, just faintly acid to the taste. To obtain perfect casts the process must be carried out very slowly, adding drop by drop to the watch-glass containing the specimen. When decalcification is complete the resulting cast should be carefully removed with a pipette, and deposited in a spot of gum on a slip. They will not stand transference with a brush without damage.

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LESSON XIII.

DRY MOUNTS.

Opaque Cells.—Place a slide in a turn-table, and run a disc of black varnish on its centre; allow this to dry. Take a piece of black paper and punch out a disc of the same size as the one on the slide, and gum it on to the varnish spot. Take a cell, either metal or vulcanite, of the required depth and fasten it to the paper disc with gold size, or black shellac cement, and put the slide away until quite dry. Now place a very small quantity of gum on the centre of the paper disc, and put the specimen into it; but take care that the gum does not extend beyond the object, or the appearance of the mount will be spoiled. When the gum has dried, put the slide into the turn-table again, and run a ring of any good cement on the upper surface of the cell, and when this has become about half dry apply a cover-glass, which must be pressed down with a needle-point until it adheres firmly to the cement all round the cell. Put the slide aside for an hour or two, and then run on a good coat of black shellac cement.

Feathers of humming-birds, eggs of butterflies and moths, small microscopic seeds, gills of many fishes, skins of fishes, skins of snakes, and transverse or longitudinal sections of stems of plants, are all mounted as opaque objects in the same manner as above. The former should be arranged in the cell in a group. The gills, skins, etc., should be well washed with distilled water and dried under pressure between two glass slips tied together with twine.

Transparent Cells.—Take a cell of the desired depth and apply a coat of cement to one side of it, and allow it to become very nearly dry. Take a slide and warm it gently over a spirit-lamp; take up the cell with a pair of forceps and place it on the centre of the slide, the warmth of which should cause the cement of the cell to melt; if not, warm a little more, and press the cell down gently

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with a needle-point until it adheres firmly to the slide all round. If the specimen is small it must be fastened in the cell with some gum, as for opaque mounts, then put it away until the gum has dried, apply a cover, and finish off as directed for opaque mounts. Leaves of plants and wings of butterflies should be mounted on a thin slide, so that both sides may be examined. No gum will be required for these specimens, but a piece of the leaf or wing should be cut or punched out as nearly the size of the cell as possible, and a thin cell should be used, so that the cover may rest on the object and keep it flat. In all dry mounts great care must be taken that all the cements used to fasten the object in position are quite dry before the cover is put on; if not, any moisture remaining will condense on the under surface of the cover and spoil the preparation.

Opaque Mounts of Pollens.—Make an opaque cell, and apply a thin layer of gum water all over its floor; then take some perfectly dry pollen and put it in the cell, shake the slide so that the pollen spreads evenly all over the cell, and let it dry. Then apply some enamel to the upper surface of the ring of the cell, and when this is about half dry apply the cover-glass.

LESSON XIV.

FINISHING OFF SLIDES.

Canada Balsam—*Quick Method.*—Take a small saucer of chloroform and a soft brush, and carefully wash away the exuded balsam. Allow the slide to dry, then place it in a turn-table and apply a coat of black shellac cement. Let this dry, then wash the slide quite clean with turpentine and apply another coat of cement.

Canada Balsam—*Exposure Method.*—Put the slide into a saucer of methylated spirit, and with a small piece of soft rag gently rub away the excess of balsam; dry the

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slide with a clean cloth, and apply a coat of any good cement.

Glycerine Jelly.—Put the slide into a saucer of cold water and allow it to soak for a few minutes, then take a penknife and carefully scrape away the jelly from the edge of the cover. Give the slide a good wash in water, and place it in some methylated spirit, which will remove the water. Dry with a clean soft cloth, and apply a coat of black shellac enamel, and when this has dried add another.

Farrant's Medium.—Allow the slide to dry for a few days, then put it into a saucer of water and wash away the excess of medium with a soft brush. Drain off as much water as possible, and, if the cover is firm enough, dry the slide carefully with a soft cloth; if not, allow all the moisture to evaporate by exposure to the air. When quite dry, put it in a turn-table and apply a coat of cement, and when this has dried add another.

Dry Mounts do not require any washing, but they should have one or two coats of any good cement.

Asphalte and white zinc cement may be used when desired for balsam or dry mounts, but they are both useless for any of the aqueous or fluid media.

A really good black enamel may be made in the following way:

Dissolve best black sealing-wax in methylated spirit until the solution is as thick as treacle, then mix this with an equal quantity of marine glue; then, if too thick, dilute with a little methylated spirit. This cement has been alluded to all through the lessons as shellac cement, and it is the best I know of for general purposes. The black enamel should be kept in a wide-mouthed, stoppered bottle. Should the stopper become fixed, just warm the neck of the bottle over a spirit-lamp, it can then be easily removed.

When a ring is being applied to a slide, the turn-table should not be run too fast, and the extreme point of the brush should only just touch the glass. A thin coat must be run on at first, then give it about ten minutes to dry.

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A sufficient quantity of cement may then be added to finish the mount, but if too much is applied at first it will overflow.

The most suitable brush for ringing slides is a sable 'rigger' No. 2 in a metal holder; it should be well washed in methylated spirit after use.

Cleaning off Failures.—During a course of microscopical work many slides will be not worth keeping, but the slips and covers are quite good, and they can be used again. When a batch of failures has accumulated, make a strong solution of Hudson's soap-powder in warm water, and place some of it in two jars. Warm the slide over a spirit-lamp, and with a needle-point push off the cover into one of the jars and put the slip into the other; let them soak for an hour or two, then wash away the soap solution with repeated changes of warm water, and finally pour away all the water and add methylated spirit; soak for a little while, and then dry with a soft clean rag.

Sometimes slips and covers have a dull, cloudy appearance, which defies all attempts to remove it. When this is the case, make up a solution of hydrochloric acid in methylated spirit (about one part of acid in six of spirit), and immerse the glasses for a few minutes. Wash away the acid with methylated spirit, and dry with a soft rag.

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PART III.

MICROTOMES: THEIR CHOICE AND USE.

INTRODUCTION.

The scope of the following chapters on the Choice and Use of Microtomes has been limited to aiding the beginner and directing his steps towards the attainment of ultimate success, and not with the purpose of adding to the knowledge of the expert worker.

An endeavour has been made to explain in the simplest manner—as far as the limited space at command would permit—the routine of practical laboratory work. Occasionally the text may appear to savour of pleonasm; for such apparent redundancy forgiveness is asked, on the ground that an explanation of difficulty has been attempted without ambiguity, instead of its being merely alluded to as if beneath notice, or, after the manner of lexicographers, omitted altogether.

It will also be noted that upon occasion a very decided opinion has been delivered. The reason for this is that the purpose of these pages is to place stepping-stones for the student in positions athwart the stream where the water may be deep. It is the hope of the writer that the stepping-stones may be found where required by the tyro at his first attempts in the fascinating art and science of microtomy.

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CHAPTER I.

Razors for Hand Section Cutting and Microtomes.

It will not be out of place primarily to clearly state that the ability to cut free-hand sections with dexterity will contribute in no small degree to good work with microtomes.

Razors.—In this connection it will be well to call attention to the best form of razor for such work.

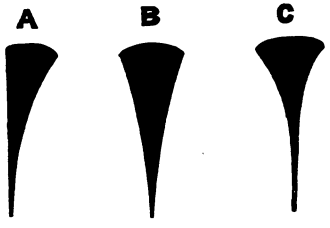

Fig. 55.—Sectional View of Razors.

Three forms are useful, and in transverse section are shown in Fig. 55, A, B, C. Quality of steel and sectional shape go hand-in-hand with form of blade. The edge should be fairly straight, and the heel rounded, not angular. The handle should be one that is capable of being grasped properly; heavy uncouth razors must be avoided, so also should one having any lettering engraved on its blade. The blade should be as broad as possible, $\frac{7}{8}$ inch being a good width. The razor must be kept free from corrosion, and the edge maintained always at its keenest by careful attention immediately after use.

The different shapes would be used as follows: A for large objects, or those that are harder than the average; B for general use; and C for small and delicate objects.

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To Set a Razor.—The time will quickly come when the edge of the razor will become notched, and in order to restore the edge a hone must be used, a strop being useless for the purpose.

Hone.—A very good hone, known as Water of Ayr stone, may be obtained for about two shillings. The size of its sharpening surface should be 9 inches by 2 inches. Water, not oil, should be used as the lubricant; and it will be found to be a good plan to allow a stream of water to flow continuously over the hone during the whole time of sharpening.

Care should be taken not to scratch the hone. Scalpels, needles, or any other such small instruments, should never be sharpened upon it. Having the hone flooded with water, lay the razor flat and diagonally upon the left of the hone in such manner that the edge of the razor is towards the right and entirely upon the hone. Draw the razor lightly but firmly edge forwards and from heel to point along the hone to the extreme right; turn the razor with a neat action of the fingers combined with a turn of wrist, its back being kept on the hone, and draw the edge forwards from heel to point as before, from right to left.

Maintain this process to and fro until the notch is removed, remembering always that the lightest pressure will secure the best and quickest results. In fact, it is impossible to hone a razor by hard pressure. When the notch is removed by honing, the edge of a razor, when carefully examined with a lens magnifying 10 diameters, should present throughout its length a straight line, with perhaps here and there a slight fringe or jaggedness standing out from the straight edge, the so-called wire edge. This may be removed by stropping. The razors with one side flat must only be honed upon the concave side, and are more difficult for the novice to sharpen than the other forms. Having dried the razor and returned the hone to its place, we now finish the sharpening by means of the strop.

The Strop.—Many kinds of strops may be purchased

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from the dealer. To a new hand, the shilling cushion strop will be quite good enough to practise on; subsequently a better strop may be obtained, and the first, although probably damaged by cuts, will be useful for sharpening scalpels. Opinions differ as to the best form of strop, but we have a personal fancy for such as approximate to the following description: A strip of wood about 13 inches long and 1½ inches wide, with a neat handle, shaped at one end, occupying about 4 inches of the length, the remainder forming the base upon which the slightly convex stropping surfaces are built (Fig. 56). Both the stropping surfaces

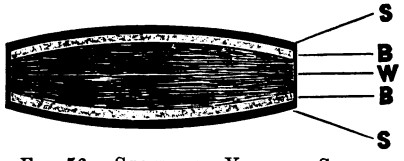

FIG. 56.—SECTIONAL VIEW OF STROP.
S, Stropping Surface; B, Bedding Material; W, Wood.

should be of the finest Russia leather. One side is usually charged with the stropping paste, the opposite surface being left bare for finishing. Some strops have the finishing side of buckskin. This we deem to be wrong for our special purpose, as the velvety buff is apt to impart a rounded edge to the razor. The longer the strop has been in service, the better will be the results obtained, provided that its surfaces have not been hacked. An old friend will have its surfaces hard, smooth, and glistening; care should be taken to keep it so. The stropping paste should be applied very sparingly at remote periods and with great care. Black paste should be avoided, as it generally contains emery powder; the red paste is usually of iron oxide. Good paste can be made by thoroughly mixing the finest jeweller's rouge with the smallest quantity of tallow. Never lay the strop down with either surface in contact with the bench. Avoid belt strops, as the result in inexperienced hands is a rounded razor edge. Holding our ideal strop in the left hand, lay the razor flat and diagonally across the prepared surface,

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and with a slight, even pressure draw it back foremost, and from heel to point, from left to right, upon the strop; turn the razor with its back upon the strop, and pass it from right to left. Maintain the process as in honing, but with the back of the razor forwards. After several dozen strokes upon the paste side, repeat the work upon the finishing side. Draw the edge of the razor between the finger and thumb or through a piece of elder-pith. If the sharpening has been accomplished successfully, a microscope having a magnification of 80 diameters will show the edge as a straight unbroken line. This microscopic examination is best made by laying the razor upon a sheet of glass. By allowing the handle to form an angle of 45° with the blade the edge will be preserved from injury. Another test is to take a hair from the head, and if the razor will cut it at half an inch from the fingers that hold it the edge is good.

Microtomes.

It is not the object of these pages to discuss every microtome which invention and ingenuity have placed within the reach of workers, neither is it any part of our intention to give the history and evolution of the beautiful instruments now obtainable. We will rather introduce the reader to a selection of well-tried instruments of variable pretensions to perfection, in order that he may be placed in a position of being able to choose from the labyrinth that or those most suited to his intended work.

Various simple forms of microtomes, such as the Cole's pattern, the Cathcart (originally made by Mr. Fraser of Edinburgh), and many others, some of which have already been referred to in previous pages of this book, appeal to workers as of practical value, according to the class of work they may undertake. But viewed from the standpoint of serious laboratory research, in which the production of the best possible results in the various departments of micro-

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tomy are to be striven for, such implements are not for a moment to be compared with the more complex and solid microtomes which we now have to consider.

Schanze's Cheap Microtome. — The cheapest of the high-grade microtomes that we can recommend is by Herr Schanze of Leipzig, illustrated in Fig. 57. This instrument has the essentials of a true microtome, and will do good work. The V-shaped tramway in which the knife-carrier slides on three raised rails is 7 inches long (or larger

FIG. 57.—CHEAP MICROTOME BY SCHANZE.

sizes may be obtained at increased cost). The jaws will hold an object $1\frac{1}{8}$ inches by $\frac{3}{4}$ inch; the object can be orientated and fixed in any required position. The micrometer screw is accurately constructed, and by means of a large divided wheel and vernier the tenuity of the section may be correctly registered. The instrument is constructed of polished steel and cast iron, and the price is only £2 15s. We shall presently describe other instruments by this maker; meanwhile we turn to the micro-

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tomes of home manufacture, and the choice is lamentably limited.

British Microtomes.—With the exception of a few instruments adapted for special work, there is nothing of British manufacture to command attention. This lack is greatly to be deplored, the scientific microscopist being dependent upon Continental workshops for that instrument of research which is second only in importance to the microscope itself.

The Cambridge Rocking Microtome.—One instrument of British manufacture and invention has won a world-wide reputation: we refer to the Cambridge rocking microtome, made by the Cambridge Scientific Instrument Company. This is, however, a special microtome, inasmuch as its mission is the cutting of small and soft objects embedded in paraffin wax, for which exact purpose it has few superiors. It is figured on p. 144.

Good as is this microtome, it is not absolutely faultless, although its failings have been magnified. Hard words have been addressed to it, yet we think had the imprecations been to the workers instead of to the microtome, there would have been less perversion of truth and justice.

A new 'rocker' has recently been placed on the market by the same makers, in which the faults which had been alleged against the old pattern have been eliminated. Respecting the tenuity of the sections obtainable with this microtome, it is probable that the instruments vary slightly. The writer's is as follows:

Forty-two turns of the micrometer screw will advance the object $\frac{9}{32}$ of 1 inch.

One turn of the micrometer screw will advance the object $\frac{1}{1419}$ of 1 inch.

As there are 224 teeth to the micrometer wheel, 1 tooth of the same will advance the object $\frac{1}{33450}$ of an inch. The sector may be set to as many as 37 teeth of the wheel, which is the maximum. The following table may be of use:

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Number of Teeth of Micrometer Wheel.	Equivalent in Parts of 1 Inch.	Equivalent in Microns.
1	$\frac{1}{33150}$	$\frac{3}{4}$ μ
4	$\frac{1}{8288}$	3 μ
6	$\frac{1}{5525}$	5 μ
9	$\frac{1}{3717}$	7 μ
13	$\frac{1}{2573}$	10 μ
19	$\frac{1}{1762}$	15 μ
26	$\frac{1}{1288}$	20 μ
33	$\frac{1}{1014}$	25 μ
37	$\frac{1}{904}$	28 μ

Fifteen μ is a very good thickness for many objects; 7 millimetres of material may be cut without readjustment.

The new 'rocker' has engraved upon it the position at which to set the sector for any required thickness. It must be admitted that sections cut by either of these two instruments are not cut in a plane, but in the arc of a circle, as will readily be seen when the see-saw motion of the upper lever is understood. This, however, is of little moment when small objects only are concerned; consequently, as before mentioned, these microtomes are only suitable for cutting small and soft objects embedded in paraffin.

The same makers have introduced an instrument upon similar mechanical principles that will cut larger surfaces absolutely flat. These are admirable machines worthy of attention, but still restricted to the paraffin method.

As the object of these pages is to aid the microscopist in his work, it is our duty to speak plainly, and to say that, to the man who can only make room for one microtome, the Cambridge 'rocker' should not be that one. But to the enthusiast who can have two microtomes a 'rocker' will prove a valuable possession for its special work. Lastly, let no one imagine that the happy possessor of a 'rocker' has but to pull the knob to and fro and produce a beautiful ribbon of sections. Success with a 'rocker' requires all the skill wanted with the Thoma, Schanze, or Minot types,

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and perhaps more. It is, unfortunately, impossible to adjust the object in any precise direction when once fixed to the plain object-holder. To remedy this defect the company have introduced an orientating object-holder. In this the cup to which the object is fixed moves in a horizontal and vertical direction by means of a ball-and-socket joint. A milled-head screw and a spiral spring control the vertical

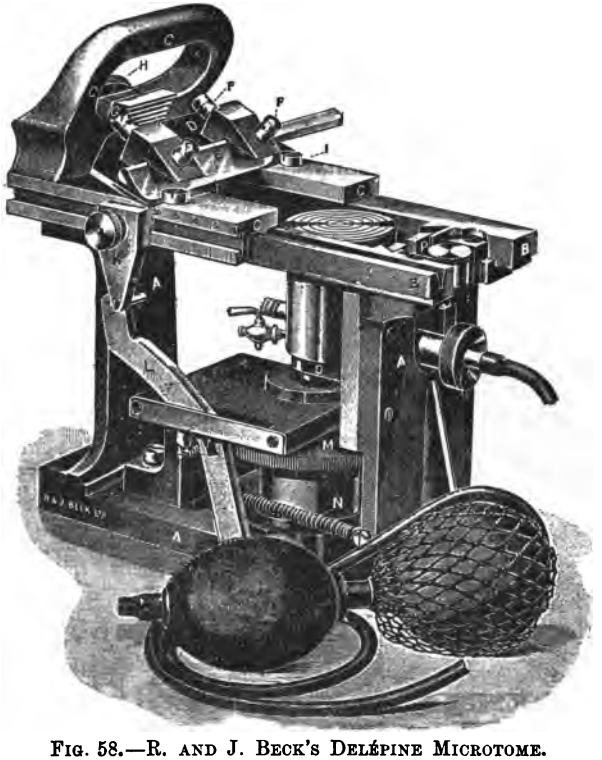

FIG. 58.—R. AND J. BECK'S DELÉPINE MICROTOME.

movement, the horizontal movement being effected by two milled nuts; these two movements, together with that obtained by rotating the object-holder, allow the object to be orientated very precisely.

Another microtome, similar in principle to those just described, has been devised by Mr. Hugh McLeake, of

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Cambridge, and manufactured by Messrs. W. Pye and Co., of the same town. It cuts sections flat, and not in an arc.

The Delépine Microtome.—Messrs. R. and J. Beck manufacture a microtome invented by Professor Delépine. This is again a special instrument, as its mission is more particularly the cutting of sections by the freezing process. It is illustrated in Fig. 58.

Beyond those enumerated, there is no microtome of importance manufactured in this country. American microtomes are mostly copies of the German instruments modified by certain alterations which may or may not be improvements.

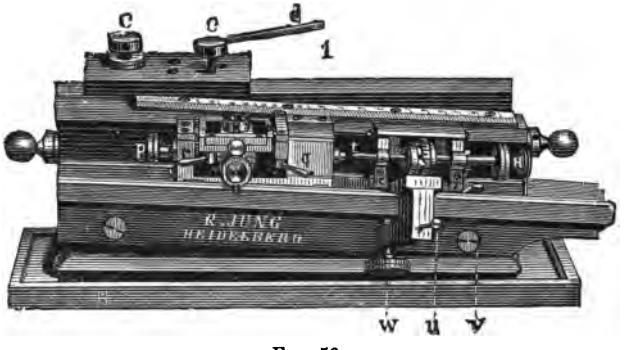

FIG. 59.

Thoma's Microtome by Jung.—A microtome of great merit, which held undisputed superiority for many years over its rivals, and still is one of the best and most useful instruments, is known as Professor Thoma's, and is made by R. Jung, of Heidelberg. This instrument, which is illustrated in Fig. 59, is constructed upon the Rivet model, the knife moving in a horizontal plane, and the object in an inclined plane. Fig. 60 presents a diagrammatical view in section of this microtome. D is its base of cast iron which stands upon the work-table on three points; C is the central support bearing the horizontal plane A on one side and the inclined plane B on the other side. The two V-grooves formed by A C and B C have each three raised sliding

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surfaces of 'rothguss' (an alloy of zinc and copper). The two heavy blocks of metal—O, the object-carrier, and K for the knife—have each five ivory points (three only are seen in the diagram), which slide upon the raised surfaces of the guides in an absolutely perfect manner. Obviously, as O is pushed up the incline so the object will be raised. The inclination of the tramway is 1 in 20, so that if the object-carrier is pushed up 1 millimetre the object must be raised $\frac{1}{20}$ of a millimetre, and the section cut will be of that thickness. To the upper

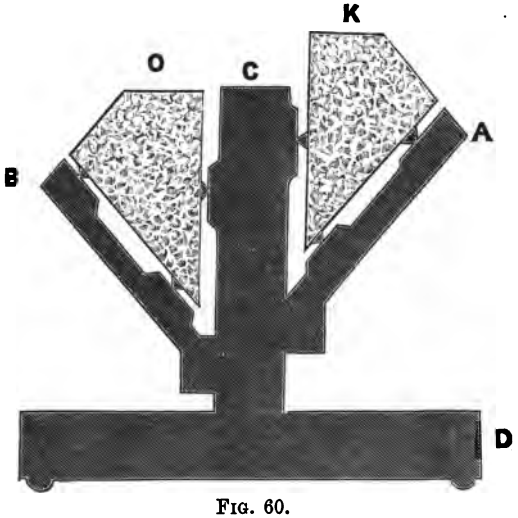

FIG. 60.

surface of the central plate C, Fig. 60, a scale is fitted, reading directly by means of a vernier elevations of the object so small as 0·015 millimetre, or 15 μ; consequently the object-holder may be pushed up its tramway by hand for sections of this tenuity or thicker. For sections of this or, in fact, any thickness, it is best to use the micrometer screw. This screw is seen *in situ* to the right of Fig. 59. The working of this micrometer screw requires a little explanation. The whole of this apparatus fits into the tramway, to which it may be fixed at any part by means of a milled-head screw below. The micrometer screw is fitted

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with a vernier in the form of a divided drum, the two halves being symmetrical, and the screw passing through the axis of the drum. Each half of the drum has cut into its periphery a set of notches, some of which are numbered as follows: 1, 2, 3, 15; these are the vernier numbers. The vernier is set by means of a steel pointer fitting into holes in the periphery of the drum. The vernier numbers, 1, 2, 3, 15, correspond to the $\frac{1}{1}$, $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{15}$ of a revolution of the drum. To set the drum to any of these portions of its revolution, the numbers upon the two halves of the drum corresponding to the portion of revolution required must be made to coincide by means of the steel pointer previously mentioned.

Suppose, for example, we bring the numbers 3 and 3 to so coincide that the notches upon each half of the drum opposite 3 and 3 are continuous. Then a spring may be set by means of a lever adjustment; when set, the knife edge of the spring presses upon the periphery of the drum, and as it rotates the knife-edge fits with a loud click into the notches that are in apposition. Thus, having set the numbers 3 and 3 to coincide, three sharp clicks will be heard for each revolution of the drum, and at each click the object will have been raised 0·005 millimetre, or 5 μ. The following table may be of use to the beginner:

When the Vernier Numbers as below are made to coincide	The Number of Clicks per Revolution will be	Each Click will give Thickness of Section as
1 and 1	1 click	15 μ, or $\frac{1}{1693}$ of 1 inch.
2 and 2	2 clicks	7½ μ, or $\frac{1}{3387}$,,
3 and 3	3 ,,	5 μ, or $\frac{1}{5080}$,,
15 and 15	15 ,,	1 μ, or $\frac{1}{25400}$,,

At first sight it would appear that only four thicknesses of sections could be cut by this method, but so cunningly are these numbers arranged that, by giving the requisite number of clicks successively, almost any thickness of section may be arrived at. Thus, for sections 10 μ thick,

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give two successive clicks when vernier numbers 3 and 3 are in apposition.

Quite a number of object-holders and various apparatus have been devised for the use of this microtome to meet the requirements of various workers. The beginner should start by procuring the Neapolitan object-holder with jaws, and subsequently obtain the other patterns as he feels the need of them. This microtome has no absolute faults; it will fulfil the various requirements of the zealous worker in a very satisfactory manner. It will be well, however, to point out inconveniences which are not enumerated in the maker's price-list. When cutting hard material or when working with a large paraffin block, the object-holder is apt to jump on its tramway at the impact of the knife, but as these conditions are not usual the objection may perhaps be shelved. A more serious complaint is that, when the micrometer screw has been turned to the extremity of its travel, it has to be turned back again, and then the whole micrometer screw apparatus has to be pushed up the inclined plane until the point of the micrometer screw again touches the polished agate of the object-carrier, against which it works. It will be readily seen that this readjustment requires very considerable delicacy in its manipulation. This matter is the greatest fault of the instrument; if the readjustment is not done perfectly, the result will be the losing of a few sections, and probably just the most important of the series.

The uninitiated will ask, 'Why lose sections?' The fact is, paraffin sections usually curl considerably upon the knife until a few have been cut. When starting a ribbon, the first few are consequently, as a rule, worthless, and the readjustment of the screw, unless very carefully done, means the starting of a new ribbon.

To unwind the micrometer screw, a piece of waxed thread passed once round its extremity and pulled in the right direction will accomplish the task in a couple of seconds.

Becker's Spengel and other Microtomes.—A micro-

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tome in no sense inferior, and in some respects superior, to the Jung-Thoma is manufactured by Aug. Becker

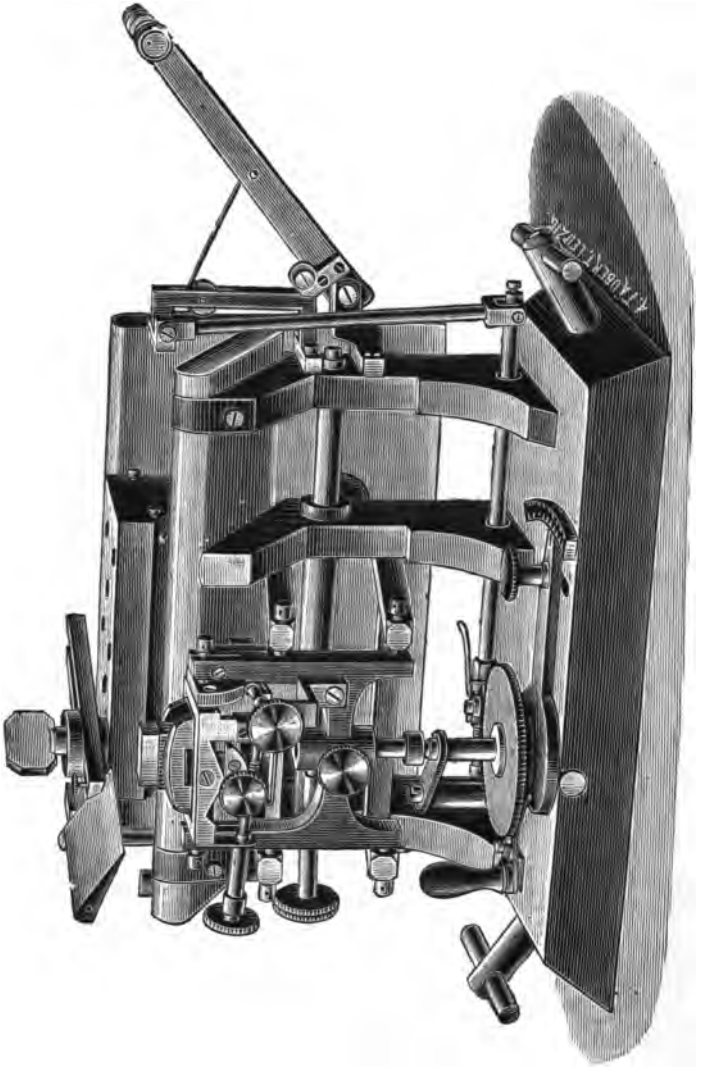

FIG. 61.—KLEINE'S AUTOMATIC MICROTOME. AN EXAMPLE OF BECKER'S WORKMANSHIP.

of Göttingen, to instructions given by Professor Spengel. It is upon similar principles to the Jung-Thoma, but

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differs in certain details. The tramways, for example, are made of glass instead of metal, so that no oil is required upon the sliding surfaces. The use of oil as a lubricant tends to the production of irregularity in the thickness of the sections. The readjustment of the micrometer screw is arranged upon a more scientific principle; the knife is worked by mechanical arrangements similar to that of Fig. 61. The price is rather lower than the Jung-Thoma. Besides this instrument upon the Rivet model, Herr Becker constructs microtomes upon the Schanze (as figured on

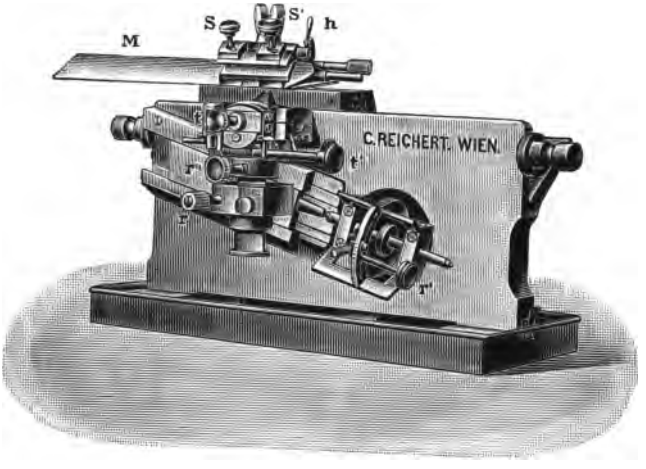

FIG. 62.—REICHERT'S MICROTOME.

p. 230) and Minot models. His workmanship is of the very highest class, leaving nothing to be desired, and his charges are moderate. The productions of this maker are therefore entitled to the consideration of microtomists.

Reichert's Microtomes.—C. Reichert, the optician of Vienna, constructs several microtomes of merit. His new continuously working micrometer screw microtome upon the Rivet model is worthy of mention, and is illustrated in Fig. 62. The object-holder is fixed upon the inclined plane, so that it does not jump when cutting hard objects. The micrometer screw works continuously, so

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that there is no necessity to unwind it. The continuous working is brought about as follows: When the screw has reached the limit of its movement, it is turned through 180°, so that its opposite extremity points towards the object-holder when it is ready for continuing the work. The two improvements to the Rivet model are undoubtedly good in principle, but Herr Reichert's improvement to the screw is not entirely advantageous. After turning the screw, the object-holder has to be pushed down the slide until it again touches the point of the screw; consequently

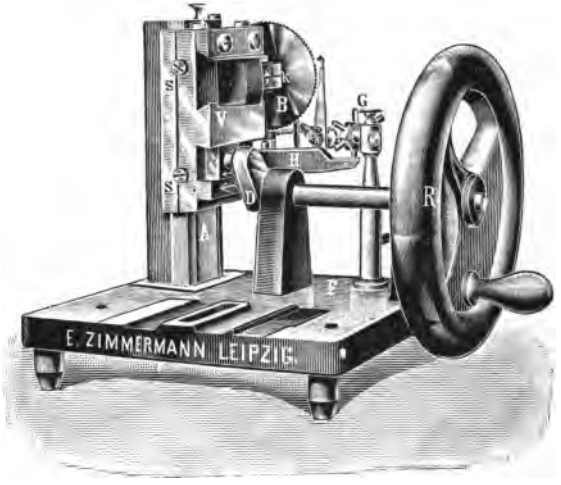

FIG. 63.—MINOT-ZIMMERMANN MICROTOME, WITHOUT KNIFE OR OBJECT-HOLDER.

a space will arise between the surface of the object and the knife-edge. This has to be compensated for by raising the object jaws by rack and pinion. Herein lies the weakness of this feature. Every time the rack and pinion has to be brought into action to elevate the object, the ribbon of sections is liable to be broken, and sections are lost before the new ribbon can be started.

Leitz's Microtomes.—E. Leitz, the optician of Wetzlar, makes a good microtome, moderate in price, and essentially the same as one by Reichert on the Schanze model.

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Sliding microtomes having an automatic feed arrangement are not always to be recommended. The automatic attachment may be so constructed as to restrict the range of thicknesses otherwise available. The cutting of sections of extreme thinness is not the chief feature to be looked for in a microtome. The writer recently had occasion to cut sections 250 μ thick; such an attainment would have been impossible with certain microtomes.

The Minot and other Microtomes by Zimmermann.—Another excellent instrument which has attained great favour is Professor Minot's microtome for the paraffin ribbon method. It is made more or less perfectly by several makers, but was introduced by Zimmermann of Leipzig. Fig. 63 illustrates this microtome without knife-holder or object-holder. It is worked by rotating the balance wheel R, whose rotary action is changed into a vertical movement of the object-slide by means of a crank action D. When the object-slide is moving upwards, a lever H strikes one of the spokes of the ratchet-wheel G, which in turn moves the large-toothed wheel B of the horizontal micrometer screw, thus advancing the object towards the knife.

There are several forms of this instrument made by Zimmermann; the most useful for general work is Model II., capable of giving sections of from 1 to 20 microns thick; that is to say, when the worker knows how to sharpen the knife to such perfection as is required for sections 1 micron thick. This remark, of course, applies to all microtomes. It must be understood that this microtome is not an all-round instrument such as the Thoma, Becker, or Schanze models. It works with great rapidity; it cuts sections up to 2 by 2 inches with exactness, and absolutely flat. The orientation of the object is easily arranged. Its only fault is that it is not compensated for wear and tear, so that in time it will require to be returned to the maker or a skilled mechanic for adjustment.

The Reinhold-Giltay Microtome.—Another microtome of great ingenuity constructed for the paraffin process is

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the Reinhold-Giltay. This instrument is fixed upon an iron stand with a wooden cover, after the fashion of a sewing-machine. Its mechanical construction is upon similar principles to the Minot, but more elaborate in detail, and its working parts are compensated for wear and tear. It differs chiefly from the Minot in the following particulars: The feed adjustment is not by an advance

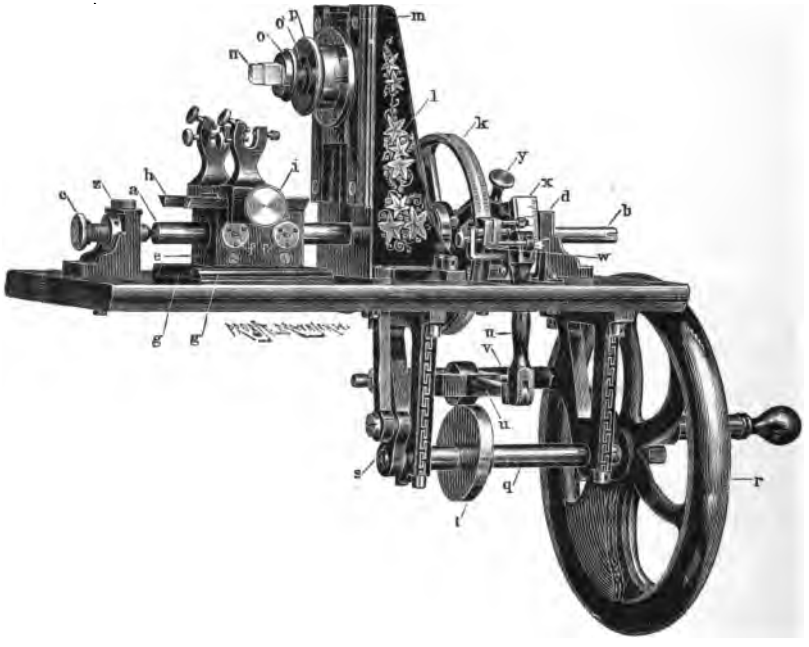

FIG. 64.—REINHOLD-GILTAY MICROTOME: MECHANICAL PART.

of the object, but by the sliding of the knife-block towards the object by means of a micrometer-screw, the movement of the object being restricted to the vertical direction. Fig. 64 illustrates the mechanical part of the microtome free from its stand. It is doubtful if for ordinary work this microtome is to be preferred to the Minot in its most complete form. As its price complete is a matter of some £26, we refer those interested to a full description by Dr. J. W. Moll

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in the *Zeitschrift für wissenschaftliche Mikroskopie und für mikroskopische Technik*, Band ix., 1892, and Band xv., 1898; also an abbreviated translation of the above article in the *Journal of the Royal Microscopical Society*, 1893 and 1899.

Schanze's Microtomes.—We have now to describe some of the instruments constructed by Herr Schanze of Leipzig, whose name has been already mentioned (Fig. 57, etc.). His microtomes are second to none in accuracy and beauty of

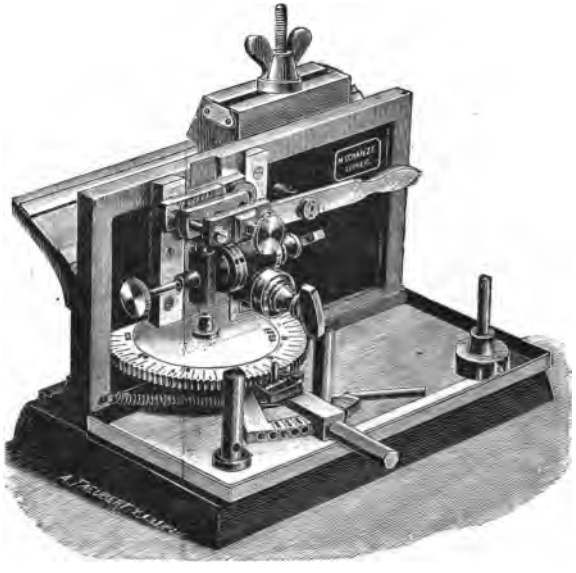

FIG. 65.—MODEL 'B' MICROTOME BY SCHANZE.

workmanship; indeed, it is doubtful if the instruments of any manufacturer excel in general utility those of Schanze. Fig. 65 illustrates his Model 'B' with focusing arrangement. The knife-carrier is a solid block of metal, having three raised ridges, which slide in the long V-groove upon three raised rails. The knife is fixed by means of the thumb-screw seen upon the upper surface of the block. The screw is movable from end to end of the block, so that the knife may be fixed in any position. The thread of the screw is

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long enough to allow of the use of the knife-holder illustrated in Fig. 66, as this form of knife (Jung's pattern) cannot be fixed to the block without the holder. A knife-holder is also supplied, which allows the knife-edge to be tilted at any usable angle. The beginner, however, should avoid this piece of apparatus.* From the under side of the knife-block an arm is extended below the tramway (not seen in the illustration), and by means of a milled-head screw the knife-block may be fixed at any portion of its

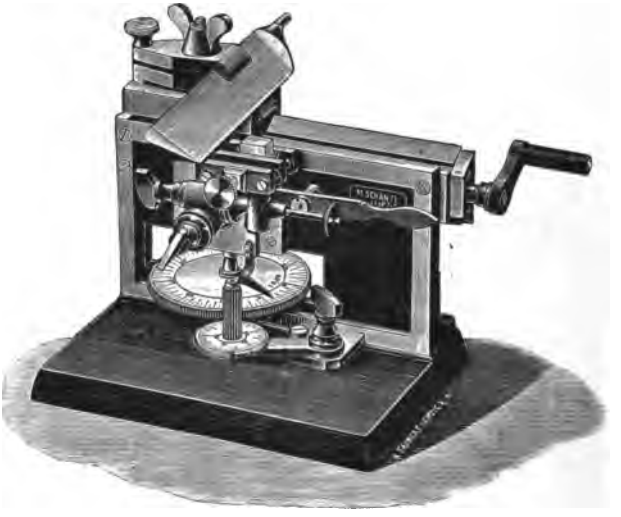

FIG. 66.—MODEL 'C' MICROTOME BY SCHANZE.

travel. The same screw, when properly adjusted, prevents the block from being pushed accidentally off the end of the tramway. The object-holder is exceedingly strong, com-

* By this advice we would not be understood to write derogatorily of this valuable addition to the microtome. A beginner should master the initial stages of microtomy before attempting to use the knife at various inclinations to the horizontal. It is useful to give the knife a little inclination when cutting thinner than usual, or when working hard material. Useful inclination may be given by placing a half-penny under the tang of the 'Henking' knife, behind the fixing screw, and another above the knife in front of the screw.

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pact, and well designed. The powerful jaws for grasping the object can be rotated, moved vertically, and finally fixed by means of the screw seen on the extreme left of Fig. 65. The object standing in an inverted position upon the base of the instrument to the right is a paraffin table, and fits into the same socket as the jaws. Besides the orientation just mentioned, the object-jaws can be moved in an arc at right angles to the tramway upon an axis governed by the thumb-screw seen to the right of Fig. 65, immediately below the long lever. The other thumb-screw immediately below and to the left of the last-mentioned governs the same kind of movement, but parallel to the tramway. By a combination of these movements the object may be easily and rapidly placed, and firmly fixed in any position that the knife-edge could reach. Added to this, if the last thumb-screw mentioned be taken off, the whole orientating apparatus may be removed and put in the reverse way, bringing the object-jaws considerably nearer the right-hand side of the microtome. Fig. 66 exhibits the orientating apparatus reversed in comparison with Fig. 65. This reversal makes not the slightest difference in the movement obtainable for orientation, and is a great advantage to the worker. When cutting with the knife set obliquely to the tramway, as in cutting by the wet method, the arrangement, as in Fig. 65, allows more travel to the knife-carrier in a direction to the right of the object. When cutting objects in paraffin with the knife set at right angles to the tramway, the reversal of the object-holder allows more travel to the knife-carrier in a direction to the left of the object (Fig. 66). The long lever to the right of the illustrations immediately under the maker's name is a coarse adjustment for rapidly moving the whole of the orientating apparatus up or down in its slide. The use of this will be described in the chapter on cutting. About 22 millimetres of material may be cut without readjustment of the object-holder. The feed arrangement is by a micrometer screw pushing up the object-carrier in its vertical slide.

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The micrometer screw is fitted with a large wheel having its periphery divided; it is also supplied with a vernier. The whole arrangement in its simplest form may be seen in Fig. 57, in which the wheel is rotated by hand to the required number of peripheral divisions. This is a tedious operation, but by the aid of an ingenious mechanism the focusing can be done without looking at the wheel, by simply moving a lever to and fro with the finger. This focusing apparatus is seen in Fig. 65. The pitch of the micrometer screw is twenty threads to the centimetre; consequently one revolution equals 0·5 millimetre. The wheel has 100 large teeth cut into its periphery, therefore an advance of 1 tooth equals 0·005 millimetre, or 5 μ. The wheel is about 110 millimetres in diameter.

The focusing apparatus consists of a sector, the arm to which the arc is fixed being secured at the centre of curvature to a longer arm upon which the sector rotates to the extent of its arc. The long arm is attached to, and rotates about, the micrometer screw, its free end forming the guide through which the periphery of the sector travels. The arc has engraved upon its upper surface a series of figures according with a certain number of teeth of the large-toothed wheel. The figures are 0·005, 0·010, 0·015, 0·020, 0·025, 0·030, 0·035, 0·040, 0·045, 0·050, being decimal fractions of a millimetre, corresponding from 1 tooth of the wheel to 10 teeth. As 1 tooth of the wheel equals 5 μ, the values upon the arc will be from 5 μ to 50 μ. The sector is set to any required number upon the arc, and fixed by means of the hexagonal rod seen projecting at the base of Fig. 65. This rod is terminated by a screw which passes through the end of the long arm into any of the ten holes at the edge of the arc, thus fixing the arm at the required position. A pawl is placed upon the long arm, which upon the forward movement engages a tooth of the wheel, but slips over the teeth in the backward movement. The movement of the sector is limited by the two uprights projecting from the base of the instrument. After an advance of the

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wheel has been made, the sector is returned to its position by means of a strong spiral spring. To prevent the wheel returning with the sector, there is an arrangement for gripping the micrometer screw, which, though sufficient to prevent any backward movement of the wheel by the action of the spring, does not hinder the forward movement. This gripping arrangement is controlled by the long steel pin seen at the base of the instrument to the right of the focusing apparatus; and this pin also fits all the other capstan-headed screws of the instrument. A silver-plated tray is fitted to the base of the microtome. All the working parts are of polished steel, the heavy casting being japanned. The weight of the instrument (as Fig. 65), with a tramway 25 centimetres long, is with its mahogany case about 32 lbs. The microtome may have other additions to its equipment. The movement of the knife-carrier in its tramway may be, if desired, effected by mechanical means. This is not at all necessary in the ordinary way, but useful for very fine work, and, as the mechanism is removable, the instrument may be used with or without its aid. The object-jaws may be further elaborated by the use of the Naples object-holder. By its means the object may be finally orientated by rack and pinion about two axes; this is useful in certain work, but not at all necessary either to the beginner or the advanced student excepting in special cases. Its great advantage lies in the fact that the axes of movement lie very close to the object, hence the object may be considerably orientated during the process of cutting without greatly altering its elevation, and the object may be raised at one end previous to cutting each section, thus giving sections thicker at one end than the other. The Naples object-holder is absolutely necessary with the Rivet model microtomes, but not so with the Schanze, as this already possesses a good orientating arrangement, as has been described.

Again, by means of another wheel to the micrometer screw below the one already described, one is able by the

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aid of a system of cogs to raise the object 1 μ at a time, or even to fractional parts of that measurement.

A microtome is illustrated in Fig. 66 upon the same model, made expressly for cutting very thin sections, from 1 μ or under up to 15 μ or 20 μ. The movement of the knife is by means of a handle seen to the right of the illustration.

In placing the merits of several microtomes before the reader, it must not be supposed that instruments by makers not herein mentioned are not worthy of consideration. We have simply given the student an insight into the construction of various instruments by a few well-known manufacturers. From a study of these pages the student will be able to select an instrument to meet his personal requirements. We might add that a second-hand microtome should be thoroughly tested before the purchase is concluded, as in careless hands a good instrument may have been easily ruined beyond ordinary repair.

Addresses of foreign microtome-makers:

C. Reichert, 24, Bennogasse, Vienna.
Aug. Becker, Mechaniker, Göttingen, Germany.
M. Schanze, 51, Brüderstrasse, Leipzig.
E. Zimmermann, 21, Emilienstrasse, Leipzig.
J. W. Giltay, Delft, Holland.
R. Jung, 12, Landhausstrasse, Heidelberg.
E. Leitz, Wetzlar.

For knives and other instruments:

W. Walb, 5, Haupstrasse, Heidelberg.

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CHAPTER II.

Accessory Apparatus.

HAVING placed the student in a position to choose his own microtome, it remains for us to indicate accessory apparatus in connection with the work of microtomy. The first consideration will be the cutting instruments. There are many patterns of microtome knives made by various makers, but the best and most useful are as follows (they are all made by W. Walb of Heidelberg):

The Selection of Knives.—1. Henking's pattern (Fig. 67) is beyond doubt the most useful knife for general paraffin cutting by the sledge microtomes. The best

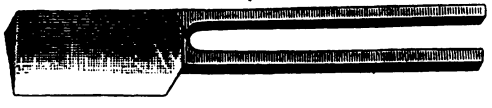

FIG. 67.—HENKING'S MICROTOME KNIFE.

size is that having the blade 6 centimetres long, and the handle 9 centimetres long. These knives, owing to their short length of blade and good-sized handle for gripping by the hand, are more easily sharpened than any other form of microtome knife, save perhaps the Jung pattern. It must be understood that the Henking knife is only suitable for paraffin or ice embedding where the knife works at right angles to the microtome tramway. Owing to their small size, they are comparatively inexpensive.

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2. **Jung's pattern** (Fig. 68), in which the blade fits into an ebony screw handle, is another useful form. It may be used for cutting at right angles to the tramway, or obliquely by means of a slicing cut—that is, if a knife of a suitable size for the microtome is selected. Owing to the shape of the handle supplied, these knives are fairly easy to

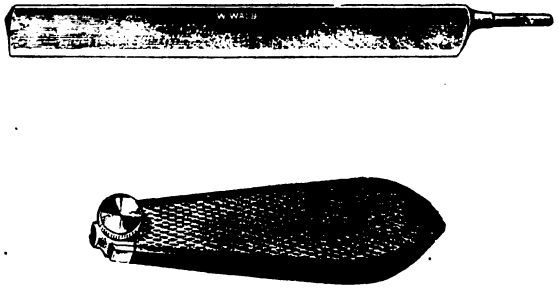

FIG. 68.—JUNG'S MICROTOME KNIFE WITH ITS SCREW HANDLE.

sharpen; they are also usable upon several kinds of microtomes, so that this pattern is an universal knife. It is fitted to the microtome in the manner indicated in Fig 66.

Weikert's and Thoma's Knives.—Two other patterns frequently used are: Weikert's, with a straight handle and

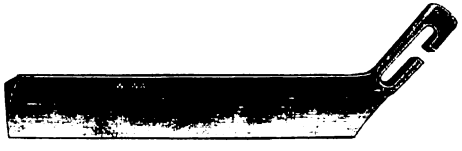

FIG. 69.—WEIKERT'S MICROTOME KNIFE WITH STRAIGHT HANDLE.

both sides of the blade concave—this is a useful tool for cutting celloidin-embedded objects under fluid; and Thoma's, or the Heidelberg pattern, with a curved handle, which has the under side flat and the upper side concave. The latter is of the same utility as the Jung pattern, but does not require a special holder, as it fits directly to the carrier of the microtome. Both these knives (Figs. 69, 70)

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we advise the beginner to avoid, for, owing to their awkward handles, they are rather difficult to hold in sharpening.

Each of the above knives, with the exception of Weikert's, is made in three degrees of concavity or hollow-grinding to their upper surface. Most microtome knives have their under surface plane.

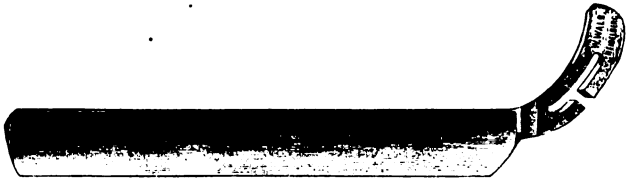

FIG. 70.—THOMA'S MICROTOME KNIFE WITH CURVED HANDLE.

In Fig. 71, A is the most hollow-ground, or thin-edged, as it is sometimes termed; it is most serviceable for very delicate objects and for the wet-cutting process. C represents the least hollow-ground, or thick-edged; it is especially valuable for hard objects, or for the ice-embedding process. B is intermediate, and is especially suitable for the paraffin method, and is the most useful sectional shape of all.

Knives Necessary.—A beginner, starting with a sledge microtome (that is, Rivet or Schanze type) having a tram-

FIG. 71.—THREE FORMS OF MICROTOME KNIVES IN SECTIONAL VIEW.

way 25 centimetres long, should have, at least, as follows: One Henking's knife—blade, 6 centimetres; handle, 9 centimetres; grinding B. One Henking's pattern—blade, 6 centimetres; handle, 9 centimetres; grinding C. One Jung's pattern—blade, 16 centimetres, with screw handle; grinding B.

A case in which to keep the knives is an absolute necessity.

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With the Cambridge 'rocker' and similar microtomes an ordinary razor is generally used; it should not be too much hollow-ground, and its cutting edge should be straight, not curved, as in the generality of English razors. If much work is intended, it is well to have more than one knife of the required pattern, so that, should the edge of one be accidentally turned, another may be in readiness, and work proceeded with without delay for sharpening.

Sharpening Knives.—To a beginner in microtomy, the one great bugbear is the sharpening of the knives. The keenness of the knife-edge is of pre-eminent importance, and the sooner the beginner realizes the fact that for the production of good work the knife-edge must be absolutely perfect, the earlier will one of his great troubles in microtomy be over.

One way out of the difficulty is to send the knife to an instrument-maker, if one can be found that understands the business. The ordinary tradesman sharpens a razor well, but is quite incapable of imparting the requisite keenness to a microtome knife unless specially trained. The right kind of microtomist will wish to learn the method of sharpening his knives, and thus be independent of the professional knife-grinder. Practice alone can bring perfection in this art; we will, however, attempt to smooth the path.

It is impossible to hold the knife during the sharpening at the correct angle without the aid of an appliance. Luckily, this is simple enough, and consists merely of a piece of steel bent to fit over the back of the knife. This slides on the knife, and is tightened up by means of a thumb-screw. Several forms are made, but this one answers the purpose extremely well; it is very strong, cannot shift its position when in use, and is cheap. In use it raises the back of the knife the requisite amount to give the edge the right bevel. Each knife should have its own blade-holder kept solely for its own use. The various sectional

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shapes of knife (A, B, C, Fig. 71) require their own special blade-holders; these should be purchased with the knives to save subsequent annoyance.

The Hone.—It is of the utmost importance to secure a good hone, which should have its upper surface about 9 by 2 inches, and commonly about an inch thick. A hone answering well enough for common razors may not be of much use for microtome knives. Some hones have their sharpening surface altogether too hard. The surface should be of exceedingly fine grain, and so soft that it may be easy to scratch it with a pin. Of course, this test should not be applied to the sharpening surface, but at the side. The yellow Belgian hone, the German razor hone, and the Turkey hone are those from which a suitable one may be selected. One worth having will cost in the size indicated about sixteen shillings. The German razor hone is obtained from Ratisbon, in Bavaria, where it is found in the slate mountains, forming yellow veins in the blue slate. The Turkey hone is obtained under similar conditions from Asia Minor. The yellow vein is commonly cut into slabs; these are secured to pieces of slate to give them strength and solidity. Or one may procure a hone so cut from the formation that the under part runs into the slate, and the upper yellow sharpening surface into the vein. It may interest readers to know that a collection of various sharpening stones is to be found in the Jermyn Street Museum of Geology, London, W.

Process of Sharpening.—Sperm-oil may be used on the hone as the lubricant, but it is more advantageous to use water and soap. One may make a lather of the best palm-oil soap with which to freely anoint the hone, or a fine stream of water may be allowed to flow continuously over the hone, and a piece of palm-oil soap rubbed frequently over its surface. With care, a knife will only require honing at remote periods, but if one has to cut hard substances which turn the edge of the knife the hone will be in daily demand. The hone must be

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used when the bevel forming the edge of the knife becomes rounded from continual stropping, or when the edge is notched or turned. A little experience will quickly teach the microtomist when to bring the hone into use. The utmost care should be taken of the hone; none but the microtome knives should ever be applied to it. After use it should be carefully cleaned and dried, wrapped up, and put away in a box. If oil is used as a lubricant, it should never be allowed to remain on the hone after use, as it has a hardening effect, and in course of time will render the stone too hard for our special purpose.

These minutiæ may appear superfluous to the uninitiated, but it is only by adherence to them that success will attend their efforts. Should the knife be notched, it will require a considerable expenditure of time to restore its edge; but in the ordinary course of setting the bevel, which forms the edge, the process is not lengthy.

Having the knife-guide adjusted upon the back of the blade, it may be mentioned that a notch made at one end of the knife-guide will enable it to be always put on the knife one way, thus maintaining its relationship to the bevel of the knife-edge. Having also the hone prepared with its lubricant, lay the knife, concave side downwards, diagonally upon the left-hand side of the hone, and draw it edge forwards and from heel to point along the hone to the right-hand side. Do not draw the knife along the hone in such a manner that its point inscribes an arc, but in such a way that its point will inscribe a straight line. Do not turn the knife as in razor honing, but repeat the above process continuously—*i.e.*, continue to draw the knife from left to right upon the hone, not allowing it to touch the hone from right to left. After a number of strokes in this manner, say fifty to one hundred for re-setting, but several hundred if a notch is to be removed, carefully dry the knife and examine its edge with a microscope magnifying about 70 diameters, when a slight fringe or jaggedness will be observed projecting from the edge of

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the knife. This is the so-called wire edge, and may be removed by grinding in the opposite direction, plane side downwards. Very slight pressure must be used in honing, the weight of the knife and the suctorial action of the lubricant being sufficient in the case of the larger knives. Patience and delicacy of touch are the factors in success. If the honing has been successfully accomplished, the edge under the microscope should present a straight unbroken line, perhaps very slightly serrated, but with nothing in the nature of a decided notch. The serrations must be removed by stropping.

The Strop.—The strop for microtome knives is obtained from Walb, and the size recommended is 36 centimetres long. It is made by securing a strip of Russia leather to a firm convex base; this is then fixed at each end to a stout wooden bow, the resulting stropping surface being convex and yielding very slightly to pressure. These strops are sent out by the maker with a thick coating of red paste upon the surface. The writer has never been able to get good results by using the strop in this condition. It may be that others are more successful with paste-covered strops, and one would naturally infer that so excellent a mechanic as Herr Walb would send out his strops in the very finest condition for microtome knives, especially so as the strops are made expressly for these knives. The practice of the writer, however, is to get rid of the red paste in the following manner: The stropping surface first receives a coat or two of sperm-oil; after this has been absorbed the strop is very carefully scraped or shaved with a keen razor—great care must be taken not to cut the strop. In this manner the major portion of the red paste is removed. Then, by application of sperm-oil and rubbing with a cloth, more of the paste is removed from the grain of the leather. Subsequently the strop receives a coat of sperm-oil, and is placed for a few days in a warm, dry place. The oil causes the red paste to work to the surface, from which it is scraped

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with a razor. After a few such applications of sperm-oil, drying and shaving, the strop is placed aside for a few days to dry, when it will be ready to receive the sharpening paste, which the writer has found to suit his purpose extremely well, and of which particulars will follow. Meanwhile the leather surface may be examined carefully in order to find the direction of the grain of the leather. When found, an arrow should be engraved on the wood each side of the strop, pointing in the direction of the grain, not against the grain. The knife should be worked in the direction of the arrow, consequently with the grain, not against it, and the arrows will facilitate this.

From these remarks it will be observed that, as there can be only one direction of grain, each side of the knife will have a side of the strop retained for its use, and in changing the sides of the knife the strop must be reversed.

Strop Paste.—The paste with which the strop is now charged is made as follows:

Diamantine No. 2, about 0·1 gramme, mixed upon a glass slab with the smallest possible quantity of sperm-oil.

Diamantine is a very fine white powder used by polishers, and made by A. Guyot-Lupold, Locle, Switzerland. It can be obtained in this country at jewellers' tool supply stores. A $\frac{1}{4}$-ounce bottle will last for years.

Stropping the Knives.—The mixture is thinly spread over the surface of the strop and rubbed in with the palm of the hand, stroking in the direction of the grain. The writer has tried many other polishing powders, but has always obtained the best results in the manner above indicated. The beginner is cautioned not to use too much of the powder, in the vain hope of putting an edge to the knife by a few strokes upon the strop. The surface of the strop will not be hard and glistening by this treatment, after the manner of the strop recommended for razors; its surface will be dull and soft, and will improve with use if its surface is not hacked, so that

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an old well-used strop is simply invaluable. Always keep the strop hanging up in one place as free as possible from dirt and damp. It is well after honing to thoroughly dry both knife and blade-holder; then, having the blade-holder adjusted upon the knife, the stropping is done as follows: Lay the point of the knife upon the right-hand side of the strop, concave side downwards, and push the knife across the strop from point to heel and from right to left upon the strop, back forwards, making the point of the knife inscribe an arc. Maintain a similar process upon the opposite side of the knife, remembering what is above mentioned respecting the grain of the strop. Never grind a knife more on the plane side than upon the concave, rather the less upon the plane side. During the stropping it will be found convenient to sit upon a low chair, and while holding the strop to allow it to rest upon the knees as well. The stropping must not be done by alternate strokes, to first one and then the opposite side of the knife, but by a succession of strokes upon one side of the knife.

Testing the Knife Edge.—When finished, the edge of the knife under a power of 100 diameters should present a perfectly straight and even edge, whilst with 250 diameters there should be a suggestion of faint serrations. These small serrations will prevent the crushing together of the paraffin sections as they are cut. Before examining the knife under the microscope, allow a drop of benzole or the like to run along the edge; then wipe with a clean soft leather, otherwise the slight amount of grease from the strop will obliterate the fine serrations; even an objectionable notch may be hidden in this manner, giving a false conception as to the real state of the edge. A fair knife-edge should cut a ribbon of sections 10 μ thick from a paraffin block having a surface 1 centimetre square without any perceptible crushing together. It will be excellent practice for a beginner to go through with this experiment until satisfaction is attained. It will perhaps take a little time, but the practical

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knowledge gained will be great, and will save many anxious hours subsequently. Acquaintance with the condition of the knife-edge necessary for good cutting is one of the foundations of successful microtomy, and can only be gained by practical work.

Infiltrating with Paraffin.—For the purpose of infiltrating the object with paraffin wax many kinds of apparatus have been devised; a favourite in many quarters is the Naples embedding bath, also several patterns made by the Scientific Instrument Company of Cambridge enjoy considerable reputation. All such apparatus are essentially water-baths, modified in form to suit the necessities or ideas of their various originators. One of the best, most useful, and most simple infiltrating devices is the ordinary double-walled, copper-drying oven of the chemical laboratory, the space between the walls being filled with water, and kept at a constant temperature by means of a gas-jet and mercury regulator with a gas-pressure regulator. The very best apparatus for this purpose is the 'Hearson' vacuum oven.

We might here mention that makeshift apparatus should be avoided if one wishes to properly infiltrate tissues. No good sections can ever be hoped for from tissues in paraffin wax that are not thoroughly infiltrated in every cell, to the exclusion of all air and clearing agent. Nevertheless, for merely embedding in paraffin, a makeshift apparatus might possibly answer.

Infiltrating Oven.—The practical worker will find the oven herein recommended to fulfil his requirements in a very perfect manner, both for infiltrating and for the many purposes for which a hot chamber is requisite. The great advantage of the plain copper water-oven over its embedding-bath competitors is that the door can be closed, and dust, damp vapours, and all other annoyances kept outside. It is also available for its original purpose in the chemical laboratory. Fig. 72 illustrates the apparatus used by the writer in its working order; the door is opened in order to show the shelves inside. A description of this

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important corner of the laboratory will be of use to the tyro. The oven is supported over the gas-burner by a four-legged iron stand; the legs fit into depressions on the zinc-covered bench. Upon the top of the oven is seen a rack, in

FIG. 72.—INFILTRATING OVEN, OPEN, SHOWING GAS REGULATOR, THERMOMETER, ETC.

which the ribbons of sections are dried to the slides (subsequently described). This rack is seen to be resting upon a sheet of plate-glass placed upon the top of the oven upon corks. This glass answers two purposes. The heat from the oven keeps it nicely warm, so that it is a convenient

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shelf on which to dry the balsam at the edges of finished slides and for other purposes. Secondly, being supported about an inch above the top of the oven, it protects from dust, objects from which the clearing agent is being evaporated in the infiltrating process.

Upon the shelf below the oven is seen the gas-pressure regulator. The use of this apparatus is to maintain an equal pressure of gas in the pipes that serve the oven. This is of great importance in places where the gas-pressure is subject to great variation. Upon the top of the oven, to the right of the slide rack (Fig. 72), will be seen the long cylindrical thermometer, the mercury bulb of which passes into the interior of the oven, not into the water.

Gas Regulators.—To the left of the slide rack will be seen the gas-regulator *in situ* with its connections. This particular regulator is Page's—a very simple and efficient instrument if care be taken in its adjustment. Reichert's regulator is in some respects preferable, on account of the easier control which it affords—by means of a screw in the mercury—over the amount of gas allowed to pass.

The regulating action of these instruments is as follows: The mercury bulb, being immersed in the water contained between the walls of the oven, responds readily to every variation of temperature. The gas is admitted into the interior of the regulator by a small hole at the end of the glass tube. If the mercury is in close proximity to the small hole, it will be obvious that any rise of temperature will expand the mercury, so that it will approximate more closely to the aperture, and thus cut off or reduce the amount of gas passing through to the burner. It is convenient to get up the heat of the oven by means of a Bunsen burner, as the pinhole burner acts slowly. When the temperature has reached the point required, as indicated by the thermometer passing into the oven, the regulator is adjusted so that any rise of temperature reduces the gas-supply. In Reichert's instrument the adjustment is brought about by turning a screw, so that the mercury may rise or

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fall to the required extent. In Page's regulator the mercury is not adjustable in this manner, but regulation is brought about by pushing the inner glass tube up or down so that its aperture may approximate the mercury. All the gas-tubes should be of metal, rubber being used only for the connections to the regulator, which should not exceed an inch or so. Rubber tubing as a permanent gas-fitting is dangerous in many ways. To the right of the oven is seen an ordinary retort stand, with glass funnel in its top ring for filtering water into the enamelled ware cup; this is for use in mounting the ribbons of sections to the slides, the filtered water in the cup being heated by the Bunsen burner below. The platform upon which the cup is resting is merely a sheet of iron about 8 by 5 inches, having an iron rod riveted to one end. The rod fits into an open boss upon the retort stand and forms a most useful hot bench. The amount of heat is regulated by the iron plate at varying elevations above the Bunsen. The pipes and two-way piece which supply gas to the Bunsen and oven will be seen in the illustration.

We have now described all the utensils necessary to be obtained, fitted up, and understood before giving any attention to the production of sections. A few other items of quite an inexpensive nature will find a more fitting place for their enumeration as we proceed with our work.

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CHAPTER III.

PRODUCTION OF SECTIONS.

Sections may be cut from many substances having a firm consistency simply by clamping them in the jaws of the microtome. Where material does not naturally possess such firm consistency as to enable it to be held in the jaws of the microtome without its being damaged, the necessary firmness may possibly be imparted to it by artificial hardening processes—such, for example, as immersion in strong alcohol for a few days. Other material from which sections are wanted may be too flabby even after hardening, or too small to be held in the jaws directly; such may be supported in elder-pith in the same manner as for hand-cutting. The elder-pith, being itself too delicate to be placed directly in the jaws, may be held between corks. For this purpose, inch cubes of the best cork may be procured from the chemical dealer. Through the centre a hole is cut with a cork-borer, subsequently the cork is halved with a keen knife, cutting longitudinally the cylinder made by the cork-borer. Each half of the cork will then have a half-cylinder upon one side; between these half-cylinders the elder-pith may be placed, and the whole fixed in the jaws of the microtome without hurt.

In cutting sections after this manner, the knife must be fixed to the carrier so as to cut obliquely to the direction of the tramway—*i.e.*, the knife must make a long slicing cut, not a square chop. The greater the obliquity of the knife the better. It will be noticed that the short Henking

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knives are not so useful for this purpose as the longer patterns.

In this method of section-cutting the object and the knife must be kept continually wet, unless the object has an oily consistency, such as some seeds, which may then be cut dry. If an object is to be cut which has been hardened or preserved in alcohol, then alcohol of the same strength must be used for the wetting; if the object has not been in alcohol, then use dilute alcohol—say, 30 per cent.—or distilled water. The wetting of object and knife is best done with a small brush, not with a pipette, a drop of the fluid on each being sufficient. Certain small and smooth-surfaced objects may be held for cutting by being cemented to a block of wood by turner's cement, made as follows:

Yellow wax	...	...	1 ounce,
Resin	...	...	3 ounces,

mixed by melting, and used hot.

The foregoing processes are essentially rough-and-ready means of obtaining sections, and although they may serve for the production of isolated or detached sections of certain tissues, such methods cannot be called microtome methods. It is impossible to cut sections thinner than about 20 μ by these processes, even when every advantage is on the side of tenuity. In the majority of substances suitable for such processes it will be found difficult to exceed in thinness 40 μ. If sections of an organ are required immediately—for purposes of diagnosis, for example—the freezing method is practically the only process available. We do not, however, recommend it for general work, as the sections obtained by this method are not to be compared with those made by the paraffin process. The freezing method, then, must be looked upon as a special one, useful in cases of emergency, but not to be relied upon as the best method for general work. If the work is likely to necessitate sections of tissues at the earliest possible moment for special purposes, then an ether-freezing apparatus may be

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purchased with the sledge microtome, or added subsequently.

Celloidin Embedding.—Another process, less used now than in the past days of microtomy, is the celloidin embedding process. This is a true microtome method, and possesses some advantage for certain objects over the paraffin method. Its great disadvantages are the slowness of the process, and the skill necessary to cut sections of such tenuity as can be accomplished with ease by the paraffin process. The sections cut are isolated—*i.e.*, you cannot cut a continuous ribbon of sections as by the paraffin method. True, the isolated sections may be mounted upon the slide continuously by a complicated process, but the majority of us live but threescore years and ten.

The paraffin method, then, is the method *par excellence* for most work. We will therefore confine ourselves to a description of this process, confident that by its means the student will be enabled to carry out the greater part, if not all, of his researches into the minute.

Collecting and Preserving.—The first attention must be given to the collection and preservation of the objects to be microtomed. These processes vary so considerably with each department and subject that it is not possible here to enter into the matter in detail. Animal histologists will find much information in Bolles Lee's 'Microtomist's Vade-Mecum'; botanists may advantageously refer to the useful pages of 'Botanical Microtechnique,' by Dr. A. Zimmermann. For instance, living organisms or portions of them must be taken at the time of robust health, and killed and fixed instantaneously by means of the substances best suited for obtaining the results desired. If you wish to make a preparation of mitosis from the embryo sac of some plant, your specimen must be thoroughly healthy and vigorous. The ovary must be cut and instantaneously placed in the fixing fluid. It is important to see that the fixative can at once reach the ovules; consequently, it is not sufficient to throw the whole ovary into the solution—the ovarian wall

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must be cut to allow immediate access of the fixative to the ovules and nuclei. If you gather your specimen and do not act at once, all the fixatives known to science applied with the utmost cunning will not preserve for you mitotic figures —will not preserve for you life-like stages in the histology of your subject, but will merely give you representations of post-mortem or pathological conditions. Remember, then, that for the representation of life-like histological structures, the utmost attention must be given to the very first process of work—viz., fixation.

Fixation.—Do not overfix, especially with the osmic and chromic salts. The length of time and the best different reagents required for fixing the various subjects can be learned by experience and from the works of reference already named. We will, however, say that, for general work on nuclei and protoplasm, the beginner will find Carnoy's acetic alcohol very useful for his first attempts. It is made as follows, and keeps indefinitely:

Absolute alcohol	...	...	350 c.c.
Glacial acetic acid	...	...	150 ,,

Use large quantities of fixative—say, 50 c.c. to object of 1 c.c. bulk. After fixing—say, for nuclei, two hours in this mixture—it is important to wash out the acetic acid. It may be well here to state that by fixing we mean the instantaneous killing and coagulation of the albuminoids and other histological elements, so that, having no time to undergo a post-mortem or pathological change, the elements may be preserved in life-like form. The subsequent hardening enables the tissues to pass through the staining and other processes without metamorphosis.

The washing out of the acetic must not be done in water, but in methylated spirit. A word on this spirit: the ordinary methylated of the shops must not be used, for this contains mineral naphtha and other substances. Spirit methylated with wood-naphtha will have to be purchased from a distillery under a permit from the Custom authorities.

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Test.—Ordinary methylated spirit of the shops turns turbid upon the addition of water, and is useless for scientific work. The other variety can be mixed with water to any extent and remain clear. We speak of this special methylated spirit as 90 per cent. alcohol.

Dehydrating and Clearing.—After fixation, the object is passed through a few changes of the 90 per cent. alcohol—say, three changes of twenty-four hours each—to rid it of the acetic and to complete the hardening. It is then passed into absolute alcohol from two to several hours, according to the size and density of the tissue, in order that it may be thoroughly dehydrated. From the absolute alcohol it is transferred to a mixture of pure xylol and absolute alcohol, the exact proportion of which is not of importance, half and half being a useful combination. In this xylol-alcohol the object must remain for several hours until partially cleared. Subsequently the clearing is completed in pure xylol. When quite clear, which may readily be seen by the transparent and hyaline character of the object, it is ready for the embedding bath. Great care must be taken that the object is thoroughly cleared by the xylol, otherwise failure will result from the inability of the paraffin-wax to mix with alcohol (see p. 269).

Final Paraffin Infiltrating Bath. — Previous to the final infiltrating-bath of paraffin, it is well to place the object when taken from xylol into a vessel containing at the bottom sufficient cold and solid paraffin to cover the object when melted. Place then the object from the last xylol-bath upon the cold paraffin, and cover the object with pure xylol; the whole should then be placed upon the top of the oven, protected from dust by the sheet of plate-glass already mentioned (p. 259). Here the paraffin will slowly melt and mix with the evaporating xylol; after a couple of hours or so a more rapid evaporation of the xylol may be brought about by placing the vessel inside the oven for a few hours. Subsequently the object is transferred to pure melted paraffin of the melting-

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point previously determined upon, in which it is kept from an hour or so to several days. The length of time required for the final infiltrating process must be found by experience, as it varies greatly in the different objects suitable for microtomic analysis. The principle to be aimed at is the complete infiltration of the entire substance by the paraffin and the elimination of all air and clearing medium. If any of the two latter substances remain in the object when the embedding is complete, all attempts at securing a good range of sections are hopeless. All your cunning at knife-grinding, all your skill and delicacy of manipulation with the microtome, will count as nothing in the impossible task of obtaining a good series of sections from imperfectly infiltrated objects. We may mention that hard objects of a dense nature take much longer to infiltrate than delicate structures of a more open character. Again, some objects may be covered with skin of such dense texture as to defy the entry of the wax*; portions of such skin must be removed or slits made through it either previously to fixing or before the paraffin-bath.

We have above indicated the method by which objects may be fixed, hardened, and passed into paraffin, in which they are perfectly preserved—being, in fact, embalmed. By this process almost any object may be preserved for years without danger of deterioration to any of its elements, provided always that the wax is kept perfectly dry and cool. It is not, however, always convenient to embed at once immediately after the fixing; consequently, a method must be sought for preserving the object in another way. After fixation and hardening of the tissues in the usual way, most subjects may be preserved indefinitely in the following mixture:

90 per cent. alcohol	...	100 c.c.	Preserving fluid.
Pure glycerine	...	100 ,,	
Distilled water	...	100 ,,	
Glacial acetic acid	...	1 ,,	

* Hearson's vacuum oven is useful in such cases.

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When it is desired to embed objects that have been in the preserving fluid, they may be placed for a few days in a considerable bulk of 50 per cent. alcohol, thence into 70 per cent. alcohol, followed by 90 per cent. and absolute alcohol, etc., completing the process of embedding exactly as if it was following immediately after fixing. The practice of keeping delicate structures intended for the microtome in strong alcohol is to be greatly condemned, shrinkage and distortion being usually inevitable.

Another important step is the final enclosing of the infiltrated object within a cube of paraffin. There are various ways of attaining this, but the very best and most certain is that known as the watch-glass method, not, however, necessarily confined to the use of a watch-glass; indeed, the best utensil to use is the shallow, flat-bottomed Berlin porcelain evaporating basin of the chemist, about 3 inches in diameter. Insist upon having a flat bottom. The basin is well rubbed all over inside with a very little glycerine, which is afterwards wiped away with a cloth, so as not to leave any visible trace of the glycerine. The basin is then placed upon the hot shelf of the retort stand (p. 259) until it is a little warmer than the melting-point of the paraffin used. Sufficient melted paraffin is then poured in to well cover the object to be enclosed. The object or objects—four or five may be done simultaneously by this method—are then placed in the wax, which is still kept fluid upon the hot shelf. By means of a hot needle arrange the objects equidistant at about ½ inch from the periphery of the basin. Of course the contour of the subjects must be known, and so arranged in the basin that the top (that is, the part at which you wish to start the cutting) is towards the bottom of the basin; with a very long object one of its sides should face the bottom of the basin, and any method which readily suggests itself may be used for noting its top and bottom for the intended cutting. It may happen that your object will not readily stand 'top' downwards in the liquid paraffin, in which case place the

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basin upon a piece of cold metal, when the lower stratum of wax will rapidly harden sufficiently to support the object, even should it be a pyramid standing on its apex.

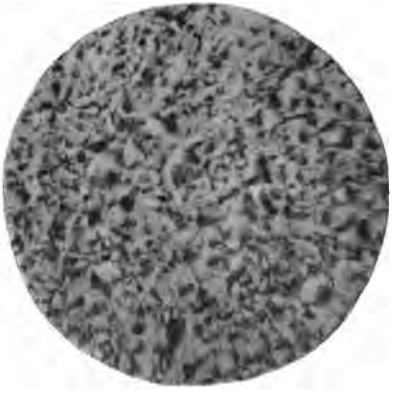

FIG. 73.—PARAFFIN COOLED RAPIDLY. (×250 Diameters.)
Very small crystals.

Immediately on placing the basin upon the cold metal, keep the object moving with a heated needle, so that it does not

FIG. 74.—PARAFFIN WAX COOLED SLOWLY. (×250 Diameters.)
Observe large crystals.

stick in the wrong direction to the cooling paraffin at the bottom. In a few seconds it will be found that the object will stand in any position in which it is placed. When

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arranged satisfactorily (the manœuvring must be done as quickly as possible), place the basin immediately in a vessel of cold water, in which it will readily float. Never

FIG. 75.—PARAFFIN COOLED SLOWLY. (×75 Diameters.)
Dark spaces, air cavities.

by any means put the basin below the surface of the water, or disaster will result by the formation of water-containing cavities in the wax.

FIG. 76.—EMBEDDED OBJECT FIXED ON WOODEN CUBE.

Cooling Paraffin.—In warm weather it is an excellent practice to float the embedding basin in such a vessel as a photographic developing dish, through which a stream of water from the tap is led by a rubber tube; or iced water

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may be used. The great desideratum is to cool the paraffin to solidity at the greatest speed.

Paraffin cooled slowly forms crystals of large and loose texture with numerous air cavities; such conditions are fatal to good section cutting. But when cooled rapidly, the crystals are small, firm, and without air cavities; the mass is homogeneous, and is in the condition sought by the microtomist. Figs. 73, 74, 75, which are photo-micrographs by polarized light of sections of paraffin, make further remarks unnecessary.

If the objects are light-coloured and not easily seen in the embedding basin when the wax is cold and semi-opaque, some method of marking their exact position must be devised. When cold, the embedded objects are extracted from the basin in the following manner: After noting the position of each object, carefully cut away the surplus wax around each, leaving each object within a good-sized cube of paraffin. Slight lateral pressure will then detach the cube from the bottom of the basin; if not, a little heat applied to the bottom, combined with the pressure, will accomplish it. The blocks of paraffin in which the objects are now embedded must be reduced in size so as to leave the object, not in the centre, but a little to one side of the block, the opposite faces of which must be cut perfectly parallel to one another. Bear in mind which is the upper side of your object, and note that air-holes, or opaque places in the cube, will necessitate re-embedding; with care, however, this will very seldom be required. The cubes of paraffin cannot be fixed immediately in the jaws of the microtome, but are cemented to the paraffin table usually supplied, and which fits into the same socket as the jaws. It will, however, be found more convenient to reject the usual paraffin table, and to cement the block to small cubes of hard wood (not cork), and then to place this in the jaws of the microtome. Fig. 76 illustrates the method. The wooden cubes may be purchased 1 cubic inch in size or larger from dealers in physical apparatus at a few pence per dozen. It is well to roughen the surface

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of the cube to which the object is to be secured, in order to give the paraffin a better grip upon the wood. The cementing of the paraffin block to the wooden cube is brought about by melting scraps of paraffin upon the surface of the wood by means of a hot metal instrument; a small screw-driver will do nicely. While the paraffin upon the wood is still liquid, the block containing the object is just warmed at the base and pressed into contact; subsequently, a heated metal instrument is run around the base of the block to complete the juncture. When the whole has become cold the paraffin block is very carefully squared, and the side which will face the edge of the knife is pared down close to the object—say, within a millimetre or two. Reference is made to this on p. 274.

The squaring of the block is easily done by eye after a little practice, or an instrument made for the purpose can be purchased. This is, however, not necessary, for if, when upon the microtome, one side is made perfectly parallel with the knife edge, and the knife is moved to the opposing side, any deviation from the parallel will be easily seen, and can be rectified by a touch of the scalpel. It is not necessary that the two sides of the block at right angles to the knife edge should be perfectly squared.

Dehydrating.—The following dehydrating apparatus will allow of specimens being passed through the various fluids without being touched, and maintained at any desired distance from the bottom of such fluids: Cut or obtain a piece of stout glass tubing of ¾ inch inside diameter in 2½-inch lengths. Make a small cork ring by means of cork-borers to fit inside the tubing; take a small circle of washed muslin, place it over the cork ring, and push the whole into the glass tube to the distance required—say, half-way; procure seven wide-mouthed stoppered bottles of sufficient size to take one of the glass tubes, and the apparatus is complete. Seven bottles are used for the dehydrating and clearing fluids as follows: Alcohols of 30 per cent., 50 per cent., 70 per cent., 90 per cent., and absolute, xylol-alcohol,

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pure xylol. The various fluids will work a long time without changing; the only thing necessary in transferring objects from one fluid to the next is to lift out the tube containing the objects, drain for a moment, and place in the next bottle. After the pure xylol-bath, the empty tube is placed upon the top of the oven to evaporate the xylol, and returned to one of the alcohol bottles ready for the next objects. The best vessels for infiltrating small objects in the oven are the small staining cells sold by opticians, consisting of a solid cube of glass with a concavity excavated upon its upper surface. The advantage of these cells is that, being thick, they retain the heat well, and the paraffin does not begin to cool directly they are removed from the oven for examination. They never upset in the oven, and they are readily cleaned.

Melting Points of Paraffin.—The paraffin for embedding should be kept in a Berlin porcelain evaporating basin with handle, spout, and lid, and should be placed in the oven a couple of hours before required for embedding. Never melt your embedding paraffin over a Bunsen or spirit lamp, as the result may be a superheated paraffin, a consequence of which is a harder paraffin than you had at first. A considerable amount of misconception exists as to the best kind of paraffin to use—that is to say, the best melting-point. All grades, however, are useful, it only being necessary to make an adjustment in the temperature of the laboratory to suit the thickness of the sections required. If your room is at 15° C., and you require sections 10 μ thick, paraffin melting at 45° C. will fulfil your requirements; but for sections 5 μ thick, with the same room temperature of 15° C., you will require a harder paraffin—say, 48° C. or 50° C.; and for sections 1 μ thick and the same room temperature the wax must have a still higher melting-point. If, on the other hand, you wish to cut sections 30 μ thick at the same room temperature of 15° C., then the melting-point of the wax should be lower—say, 42° C.—or you may raise or lower the temperature of the room to suit the melting-point of

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the paraffin and the thickness of the sections required. If the paraffin is too soft for the temperature of the work-room and thickness of sections, then the sections will probably crumple together; if the paraffin is too hard, then the sections will curl or roll upon the knife. A little rolling, however, of the first few sections is of no importance; as the first two or three are cut, they should be unrolled by the aid of two 'crow-quill' sable hair-brushes slightly moistened in the lips, then, other matters being favourable, the ribbon of sections will come away satisfactorily. You may cut a ribbon of sections without the slightest curling, when isolated sections cut at the same time and under the same conditions will curl beyond hope.

Cutting Sections.—In the remarks that follow upon cutting, we shall presume that the Schanze microtome (Fig. 65) is being used. The space at command does not admit of entering further into the working of the various instruments previously described, so that the remarks must be confined to one, although applicable to any.

In the first place, the Henking knife must be secured square with the tramway; this is easily brought about by having a few lines scratched upon the upper surface of the knife-carrier at right angles to its direction of movement. The handle of the knife can then be made to coincide with one of the lines; the blade will then be sufficiently square for practical purposes. The object has to be orientated into correct position. If the object has to be exactly vertical to the direction of the knife movement, first square the object by eye with one of the vertical fittings upon the body of the microtome. It may then be squared in the other direction by placing the edge of the knife immediately over the object and close to it, and examining the reflection of the object upon the under surface of the knife. When the object and its reflection form a straight line, the object must be plumb. Orientating operations are facilitated by the free use of the long lever-arm, which moves the object rapidly in a vertical direction without giving need-

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less wear to the micrometer screw. Having arranged the orientation, the two surfaces of the paraffin-block must be brought absolutely parallel to the knife-edge, as previously mentioned (p. 271). A lens will be found of great assistance in this operation, especially if it can be supported over the object so as to leave both hands free. The knife-block with knife attached should now be removed from its guides, in order that the tramway may receive a little oil. One drop of sperm-oil should be rubbed over the three raised surfaces, and then wiped off with a wash-leather kept for the purpose. Oil in quantity or dust upon the slides will give no end of trouble. The micrometer screw and the focusing apparatus, described on page 246, should now be adjusted, and everything is ready for cutting. If the embedding has been performed in the manner recommended (p. 268), there will probably be a slight thickness of wax to be cut through before the object is reached. This is an advantage, as it allows a ribbon to be fairly started before the object is cut into.

In manipulation by hand, the knife-carrier is lightly but firmly held before and behind, by the thumb and forefingers, and moved gently, but rather quickly, to and fro. The knife need not be made to travel the whole length of the tramway, two inches or so in front of the object to half an inch beyond will be sufficient. Previous to the advance of the knife, pull the focusing-lever towards you so as to raise the object the required distance. In releasing the focusing-lever, do not let it run back so as to strike the stop-pin a smart blow, but guide it back with the hand, otherwise tremor will be imparted to the whole microtome, to the detriment of the sections, especially to the very thin ones. As previously mentioned, the few first sections will probably curl upon the knife; they may be unrolled in the manner indicated (p. 274). If they cannot be unrolled they must be removed, the knife-edge carefully cleaned, and another start made.* The cleaning of the knife-edge is easily

* Curling upon the knife is in consequence of the paraffin being too hard for the thickness of sections. To remedy this, cut thinner

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accomplished by the aid of a drop of xylol, or the like, applied with a brush and wiped off with a clean soft leather below as well as above. It is very important to keep the knife-edge free from stray particles of paraffin; the presence of such will cause the ribbon to become crooked, will facilitate curling, and, what is worse, will cause longitudinal slits in the sections. If you find a ribbon of sections which began well, suddenly become afflicted with one of the maladies just mentioned, cleaning the knife with xylol will often remedy matters. Another cause of failure is from the knife-edge having become turned with a hard object; to remedy this, bring another portion of the knife into action. Bear in mind the failures likely to result from bad knife-sharpening. With sledge microtomes the ribbon should be cut into suitable lengths as it is given off. It is well, however, to allow the ribbon to fall over the back of the knife until its end approaches the base of the microtome, then a length should be cut off sufficient to be afterwards cut into two or three lengths for finally mounting.

Treatment of Ribbon Sections. — The best size of cover-glass for 3 inches by 1 inch slides is $1\frac{1}{2}$ inches by $\frac{7}{8}$ inch. Supposing, then, that your sections are of such size that three rows of six in a row will be well covered by the cover-glass when mounted, then you cut off from the end of the ribbon with a pair of scissors twelve or eighteen sections, and keep them carefully under cover until all are cut, taking care to remember at which end is the first section, in order that the proper sequence may be maintained in mounting. Be careful when cutting off the lengths to leave five or six sections intact upon the knife, because, if you take away all of them, when cutting is resumed the first few sections will curl again and be lost. It is best to cut all the sections as rapidly as possible when they have been started. If the microtome is left for a

sections, or place a lamp or Bunsen burner close to the microtome for a few minutes, or alter the inclination of the knife (see p. 244).

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few minutes in the midst of cutting, it will sometimes happen, owing to effects of expansion and contraction, that upon resuming the cutting the first section will be thicker or thinner, or perhaps even missed altogether.

In the process of cutting, the sections will probably become slightly crumpled or compressed together longitudinally, so that previous to fixing upon the glass slips they should be stretched in the manner to be described immediately. For the preservation of the correct sequence, the calculated number of slides should be prepared by having the designation of the organ written with a diamond upon one end of the slip, together with the number of the slip, and indicating the direction in which the consecutive sections run by an arrow. This enables the side on which the sections lie to be at once distinguished. As a support for the slips, the sloping water-colour palettes will be found useful.

Mayer's Albumen.—Each slide then receives a small drop of Mayer's albumen; this is rubbed over the surface of the slide with a clean finger, and finally almost cleaned away with another finger, leaving a scarcely visible film. Mayer's albumen is made as follows:

White of a perfectly new-laid egg ...	25 c.c.
Pure glycerine	25 ,,
Sodium salicylate	0·5 gramme.

Place in a perfectly clean bottle, and allow to stand for a week or more, with an occasional vigorous shaking, until the soda is dissolved. Subsequently, filter into a clean and dry bottle. It will take a week or more to filter, and must be protected from dust meanwhile; the solution will then keep indefinitely: its use is to stick the sections to the slide.

Stretching Ribbon Sections.—To stretch the sections take a strip of ribbon sufficient for one length—say, six sections—and float them upon warm water contained in a long and narrow glass dish, about 3 inches by 1 inch, remembering at which end the first section occurs. This also will prevent the ribbon turning about upon the surface of the water, in which case, after it had per-

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formed a few evolutions, one end would not readily be known from the other. Subsequently, hot water is carefully conducted to the bottom of the dish in which the ribbon floats, by means of a pipette. Take care not to introduce air-bubbles through squeezing the teat too vigorously. Upon application of hot water the sections will open out in a wonderful manner, and ribbons apparently crumpled beyond hope may be restored by this simple means.* The amount of hot water that the floating sections will bear may readily be seen by the paraffin gradually losing its white opacity as its melting-point is approached. Filtered distilled water should be used.

Mounting Ribbon Sections.—Having the stretched ribbon floating upon the water, take the properly-prepared slide (remembering as to the sequence of sections) and, placing it under the ribbon, float the ribbon into position upon it. It is sufficient to place one side of the slide in the water under the ribbon, which may then be held in position with a brush whilst the whole is lifted out of the water. Any required number of strips of ribbon may be placed side by side to fill up the slip, after which all of the superfluous water possible should be removed from the slide, and it should then be placed in the rack sections downwards to dry at room temperature, or stand the rack upon the top of the paraffin oven. On no account should any further progress be attempted until the sections are thoroughly dry. They will keep indefinitely if dry and free from dust. Manipulation of the ribbon is easily done by means of a crow-quill sable hair-brush, and a wide, thin and flat bone chemical scoop.

In pushing the sections from the bone scoop on to the surface of the water for stretching, care must be taken not to introduce water between the ribbon and the bone, or failure will result from the sections sticking to the instrument. These manipulations are easily and

* Do not rely upon this method for making good the results of bad knife sharpening.

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quickly done, although difficult to describe exactly. The sections will often stick to the slide without the aid of the Mayer's albumen, simply by the drying of the water alone. To make doubly sure, it is as well to combine the processes, especially should the cut material be deficient in albuminous matters, or if that substance has been over-coagulated in the process of fixing.

Finishing Ribbon Sections.—Glass slips and covers are readily cleaned by immersion for a day in strong sulphuric acid; they should be put in singly. Subsequent washing for a few hours in running water will rid them of all trace of acid; then they are stored in 90 per cent. alcohol until required for immediate use. When sections that have been stuck to a slide are to be finished off, the paraffin is removed by placing the slide upon a hot bench for a few moments until the wax is just melted, when it is blown to one end of the slide and wiped off. The hot slide is at once placed in pure xylol to rid it of the remaining paraffin. From the xylol it is transferred to 90 per cent. alcohol for a short time to rid it of the liquid hydrocarbon and to coagulate the Mayer's albumen, insuring thus the adhesion of the sections to the slide during the subsequent operations. The xylol may be used many times over until it becomes saturated with paraffin, in which case the first alcohol bath from xylol should consist of hot alcohol—say, about 40° C., in order to rid the sections thoroughly of any paraffin that may be brought away from the paraffin-saturated xylol. The passing of the mounted sections through the various fluids previous to the final inclusion in Canada balsam is most readily accomplished by means of grooved porcelain troughs made with five grooves, and holding ten slides, if pairs are placed back to back. At least nine of these troughs should be procured, and fluids kept in them as follows:

Xylol; 90 per cent. hot alcohol, then through diminishing strengths of alcohol—90 per cent., 70 per cent., 50 per cent., 30 per cent. and finally distilled water; two troughs remain for the staining processes. A plain glass cover

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placed over each will keep out dust and prevent evaporation, as the fluids in each will serve for many sets of slides. The 3 inches by 1 inch glass troughs are also very handy, especially for certain staining processes, as they may be placed under the microscope for examination during operations; they will also hold several slides of sections, in which case the sections should be downwards, and a small strip of glass placed between the slides at one end. If your object has been stained *in toto* previous to embedding, then, after the xylol and 90 per cent. alcohol bath it only remains to clear in xylol-phenol and enclose in balsam in the usual way. If you pass sections stained *in toto* from the paraffin dissolving xylol-bath into balsam, without the intervention of the alcohol-bath, failure will almost certainly result as a consequence, first, from a trace of paraffin, and, secondly, from the Mayer's albumen being carried into the balsam and causing cloudiness.

It will be generally found by the beginner that staining sections upon the slide is more under his control than staining in bulk, and will yield him more information respecting staining in its scientific meaning as distinguished from mere dyeing. In scientific staining we aim, not at the mere colouring of tissues to render them more beautiful, but to differentiate structure by colouring the various elements differently. The æsthetic microtomist will aim at a combination of the scientific with the artistic, in so far as there be no departure from the erudite rendering of nature.

In brief, the theory of staining is to colour certain elements of a tissue—otherwise, perhaps, difficult of observation—of a different colour to surrounding elements, by taking advantage of the affinity of certain elements for certain stains, bearing in mind the knowledge gained by a previous study of the subject, and knowing exactly what it is wished to differentiate. Sections that are to be stained upon the slide may be stained in alcoholic solutions of stains of varying alcoholic strengths, or in aqueous solutions, or in both.

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The advantage of staining solely in alcoholic solutions is that the sections have not to be passed from strong alcohol into water, water acting injuriously upon some tissues, but not affecting the majority of subjects in the least if care be taken to make the transfer from strong alcohol to water as gradual as possible by the intervention of gradually decreasing grades of alcohol. Passing from water to strong alcohol should be through gradually increasing grades of alcohol; hence the use of various grades of alcohol recommended to be in readiness in the grooved porcelain troughs and elsewhere. The various grades of alcohol are prepared sufficiently correct enough for practical work as follows:

For 30 per cent. alcohol—take 90 per cent. alcohol, 300 c.c.; add distilled water, 620 c.c.

For 50 per cent. alcohol—take 90 per cent. alcohol, 600 c.c.; add distilled water, 420 c.c.

For 70 per cent. alcohol—take 90 per cent. alcohol, 800 c.c.; add distilled water, 200 c.c.

For 90 per cent. alcohol—methylated spirit as purchased (see p. 264).

Rectified spirit of the British Pharmacopœia is 84 per cent. alcohol; 98 per cent. alcohol is the usual absolute alcohol.

The formulæ above given are convenient quantities for half Winchester bottles.

We do not propose to give the endless number of formulæ for mixing stains; such in abundance, together with directions, will be found in the three following works: 'Botanical Microtechnique,' by Dr. A. Zimmermann; 'The Microtomist's Vade-Mecum,' by Mr. A. Bolles Lee—both previously mentioned; 'Methods and Formulæ,' by Mr. P. W. Squire, an invaluable little work.

We must point out that the most simple formula will often prove the best friend to the student. A certain amount of quackery exists among microscopists, as in other persons, and there are to be found men willing—nay,

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anxious—to add almost any substance to their stains without rhyme or reason, veritably shooting in the dark in the vain hope of bringing off something good. This is more especially the case in many of the innumerable carmine and hæmatoxylin mixtures that have from time to time been circulated.

For general nuclear and cytological work a simple hæmatoxylin, counterstained with Bismark brown, will be as useful and easy to work with as a beginner might wish. We would not be understood to extol these two stains above every other stain; there are undoubtedly better, but for the beginner they are the most easily worked stains, either singly or in conjunction, that we know of for cytological work in general. We append formula:

P. Mayer's Hæmatoxylin Solution (Stock Solution).

A. Dissolve with gentle heat.

Hæmatein, or hæmatein-ammonia	0·25 gramme.
Distilled water	1 c.c.
Absolute alcohol	12 c.c.

B. Dissolve with gentle heat.

Alum	12·5 grammes.
Distilled water	250 c.c.

Add *A* to *B*, filter, and add a scrap of thymol, which will keep it good for several months.

Bismark Brown Solution:

Bismark brown	1 gramme.
Absolute alcohol	40 c.c.
Distilled water	160 c.c.

The order of this staining process is as follows: From the xylol-bath pass your sections through the various grades of alcohol—a few minutes in each—into distilled water. If placed from alcohol into the hæmatoxylin solution a deposit will be formed upon the surface of the sections which

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will ruin them. From distilled water transfer to the following:

Stock solution hæmatoxylin, as above ...	5 c.c.
Distilled water or alum-water	95 c.c.

Instead of the plain distilled water it is better to dilute the stock solution with weak alum-water, of which a stock solution may be made up as follows:

Alum	10 grammes.
Distilled water	1,000 c.c.

The strength of the solution must be varied to suit the work. An endeavour should be made to so gauge the strength that the solution will take several hours to thoroughly stain the nuclei without affecting the cytoplasm too deeply.

When stained, transfer to tap-water five minutes, then place in distilled water a few minutes. If accidentally stained too deeply, alum water is the remedy. If the process cannot be completed at this stage, the hæmatoxylin-stained sections may be kept in 50 per cent. alcohol for a time; water will bleach them in a few hours. From distilled water pass the sections into Bismark brown—full strength as given in the formula—for about ten minutes. Thence pass gradually through the various grades of alcohol to 90 per cent., say, ten minutes in the 30 per cent. and 5 minutes in the subsequent grades.

From 90 per cent. alcohol clear the sections in xylol-phenol by placing the slide, section downwards, in a 3-inch by 1-inch trough containing a little of the clearing agent. Having first wiped off the superfluous alcohol, the slide should be supported at one end by a strip of wood, otherwise the sections may be damaged by contact with the bottom of the trough.

XYLOL-PHENOL FORMULA.

Pure xylol	100 c.c.
Absolute phenol	100 grammes.

Dissolve with gentle heat, and filter.

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This is the best all-round clearing agent known; it clears from 90 per cent. alcohol in one minute; it does not shrink tissues, nor does it affect stains.

Drain off the clearing agent, place a little ridge of xylol-balsam upon the middle of the slide from one end of the sections to the other, gently lower the cover-glass, arrange it into position with needles, and place the mount upon the glass shelf above the oven, or even upon the top of the oven, to evaporate the xylol; subsequently finish.

Xylol-balsam Formula.

Fill a 6-ounce corked bottle with *dried* balsam.
Add sufficient pure xylol to barely cover the balsam.

It will be ready for use in a week or so. Balsam that has been properly dried, free from oil in a sand-bath, must be used; the ordinary Canada balsam of commerce will never dry if used.

The rationale of the above double-staining process is as follows: The nuclei are far more greedy for the stain than the other elements of the organ; consequently the nuclei are stained first with a stain that does not affect the cytoplasm too deeply, and which does not readily wash out in either water or alcohol during the subsequent operations. Hæmatoxylin fulfils this requirement in a high degree. Then it is necessary to counter-stain the remaining elements with a stain having the following characteristics: First, it must give good contrast in colour to the hæmatoxylin; second, it should have no tendency to wash out or deteriorate the first stain; third, it must stain quickly, and not be liable to wash out in the dehydrating alcohols. Bismark brown fulfils our second requirement.

Of course glycerine-jelly and other mounting media may be used instead of balsam. Such will necessitate a slightly different method of procedure after the alcohol baths No. 15.

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Recapitulation of pages 264-284.

1. Perfectly fresh and healthy material.
2. Fix with your utmost ability, but do not overfix.
3. Wash out fixative, bearing in mind that the choice of a washing fluid must be governed by the fixative used.
4. Harden.
5. Dehydrate. If from an aqueous washing solution, then in gradually increasing grades of alcohol.
6. Semi-clearing fluid.
7. Clear thoroughly.
8. Xylol-paraffin bath.
9. Infiltrate in pure paraffin wax; do not have the temperature of the oven higher than the melting-point of the paraffin.
10. Embed the infiltrated object, and cool the wax as quickly as possible.
11. Prepare the blocks containing the object, and cement to cubes of wood.
12. Prepare microtome for cutting, orientate the object perfectly, square the block, and cut.
13. Prepare the slides, stretch the strips of ribbon, and float them into position on the slides in proper sequence; put away to dry.
14. Clear away paraffin from sections by heating and a xylol bath.
15. Alcohol baths.
16. Staining (not dyeing).
17. Dehydrating baths.
18. Clearing in xylol-phenol.
19. Enclose in xylol-balsam, glycerine jelly, or other medium, and finish.

NOTE.—It is occasionally useful to make a preliminary examination of a spare section. Mounting in clove-oil, in which eosin or other stain has been dissolved, will clear away the paraffin and stain at the same time. Heat will accelerate the action.

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